MORPHO-PHYSIOLOGICAL RESPONSES AND PERFORMANCE OF CHICKEN FED ACTIVATED CHARCOAL – AGED PALM SAP (ACAPS) SUPPLEMENTED DIETS

BY

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CERTIFICATION

This is to certify that this thesis titled "Morpho-physiological responses and performance of chicken fed activated charcoal - aged palm sap (ACAPS) supplemented diets" was carried out by Ohanaka Albert Uzochukwu Chinenye (Reg. No. 20164999578), in partial fulfilment of the conditions for the award of Doctor of Philosophy degree (Ph.D) in Reproductive Physiology, in the Department of Animal Science and Technology, Federal University of Technology, Owerri.

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DEDICATION

This thesis is dedicated

to God Almighty for his infinite mercy upon my life, my wife, Dr. Judith Ohanaka, and son Chiadikanma Louismaria Ohanaka, my parents, Mr. and Mrs. G.E. Ohanaka and Rev. Sr. Roselucia. C. Oparaji, for their support, love, care and encouragement towards the completion of this programme.

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ABSTRACT

The aim of this study is to utilize a feed additive supplement developed from a combination of activated charcoal and aged palm sap (ACAPS) for the improvement of the performance of laying hens and broilers. Pig dung, palm kernel shell (PKS), and bamboo chips blended respectively in a 4:3:3 ratio was carbonized to produce activated charcoal (AC) as a potential poultry feed additive. Thereafter, the AC was blended with raffia palm sap that was aged for 3 days in a ratio of 7:3 to produce an activated charcoal-aged palm sap (ACAPS) product as a second potential poultry feed additive. The efficacies of the AC, and ACAPS as feed grade additives were tested on laying hens, and broiler chicken. In the layer experiment, one hundred and fifty (150) Isa brown laying hens aged fifty (50) weeks were randomly assigned to five (5) groups (L1-L5) of 3 replicates with 10 birds each in a completely randomized design (CRD) experiment that lasted for 12 weeks. Five experimental diets containing either 0.00, 0.50, and 1.00% AC or ACAPS were formulated with zero AC as the control. Similar dietary supplementation of AC, and ACAPS (0.00, 0.50, and 1.00%) were used to produce the broiler starter, and finisher diets. Again, 150 Abor acre day - old broiler chicken was randomly assigned to five groups (B1 – B5) of 3 replicates with 10 birds each in a completely randomized design (CRD) experiment that lasted for 8 weeks. Data collected from the layer experiment included laying performance, egg quality, haematological, serum biochemical, and reproductive organ characteristics, while for the broilers, data on the growth performance, carcass characteristics, and economics of production were also collected. Results from the physico-chemical evaluation indicate that AC was mildly alkaline (8.49), high in carbon content (75.35%) and low in ash content (13.13%). The inclusion of the AC in the layer and broiler diets made the diets more water absorbent, increased carbohydrate, and metabolizable energy values, but decreased the BD, crude protein (CP), and other proximate values of the diets. The mineral ratios of the diets such as Ca/P, Na/K, and the dietary electrolyte balance (dEB) increased with increasing AC inclusion in the layer diets. The 1.00% AC and ACAPS supplementation resulted in 8.28, and 12.84% increase in hen-day production over the control value, and reduced loss in weight of the laying hen. All haematological, serum protein, and electrolyte values were significantly ($p<0.05$) decreased with increased inclusion of AC, and ACAPS supplementation, while the ovarian, and large yellow follicles (LYF) weights were increased. Litter odour and fly infestations were reduced in the pens housing the charcoal treated birds. The performance results of the broiler study showed that the birds fed the 0.5% AC (B2) recorded significantly higher ($p<0.05$) final weight, and weight gain, followed by the control, and B4 (0.5% ACAPS) groups after 7 days of feeding. After 28 days of feeding, the birds fed the B4 (0.50% ACAPS) diet recorded significantly higher final body weight, and weight gains ($p<0.05$) than the birds fed the B3, and B5 (1% AC and ACAPS) diets. Similar growth pattern was observed on the 42, and 49 days of age. However, on the 56th day, the B2 (0.50% AC) had averaged similar ($P>0.05$) performance with the B4 and both had superior performance indices than the rest of the group. No significant differences were observed in the carcass characteristics of the birds. The blood parameters improved progressively with charcoal addition in the broiler diets. The optimal supplementation levels for AC and ACAPS in layer diets were at 1.00% levels because of improved egg laying, growth performance, and eco-friendly poultry production environment. The optimal levels of AC and ACAPS supplementation in broiler diets stood at 0.50% levels beyond which it comes less tolerable and impacts on broiler performance. Therefore, the supplementation of ACAPS at 0.50 and 1.00% in broiler and layer diets respectively is recommended as feed grade additives for optimal performance in poultry production.

Keywords: Activated charcoal, palm sap, layers, broilers, poultry feed.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Intensive poultry production accounts for about 21% of the poultry industry in Nigeria (FAO, 2019) and has become a major source of animal protein supply to her citizens. The industry contributes about a quarter of Nigeria's agricultural gross domestic products (AGDP) to her economy (The Cable, 2019; NEA, 2020). However, the growth, and sustainability of the industry has been greatly constrained by the increasing cost of feeds, and feedstuffs due to the shortages in grains supplies for animal feed production. In the face of this competitive raw material demand, and the increasing human population, researchers have suggested the adoption of non-conventional feed resources (NCFR) not directly utilized by man as feed ingredient in poultry nutrition (Makkar, 2016; Tona, 2018), to reduce cost of feeds and animal protein products. Despite decades of research on NCFR, the acceptance or incorporation of such ingredients into the poultry feeding system has been limited by a combination of factors that border on quality, availability, and productivity (Iyayi, 2009; Bhuyan *et al.*, 2020). This is due to their high content of fibre, limited available nutrients, anti-nutritional properties, and low digestibility, which limits performance and productivity in birds (Adeola, and Olukosi, 2009; Katoch, Tripathi, and Sood, 2018). It has been suggested that with proper processing, and treatment using functional additives; agricultural by-products could be utilized as feed ingredients in poultry production (Abdelnour, El-Hack, Mohamed, and Ragni, 2018; Bhuyan *et al.,* 2020).

Additives such as minerals, vitamins, methionine, probiotics, and enzymes inclusions in poultry feeds is widely used all over the world, and they are believed to improve the quality of feed, nutrient digestion capacity, and utilization (Abd El-Hack *et al.,* 2020). Despite the established advantages associated with the use of feed additives in poultry feeding, their high costs unfortunately, make them unavailable, and not readily accessible to small-holder farmers that make up the bulk of poultry farmers in low-income countries like Nigeria (Ohanaka *et al*., 2018b). It would be therefore advantageous to resource-poor poultry farmers under small-scale poultry farming conditions, if cheap, and readily available feed additives could be identified for inclusion in the diets of chickens. Some of these additives could be produced from locally available agricultural residues (ARs) and agro-wastes (Okoli *et al*., 2014; Ohanaka, Duruanyim, Etuk, Uchegbu, and Okoli, 2017).

For example, the processing of pig dung and other agro-waste for biofuels, and the use of the ash produced from their combustion as livestock mineral additives could help in reducing the dung overflow in farms across Nigeria, and possibly help decrease the nuisance of manure piles and environmental degradation (Iregbu *et al*., 2014). The controlled combustion of pig dung blended with fire accelerants like palm kernel shell and bamboo wood at appropriate blends have been shown to improve the combustion value of the dung to produce a biochar, charcoal or ash that could be applied to livestock production (Iregbu, 2014).

Specifically, ash derived from AR combustion could form a readily available source of mineral supplement in poultry ration (Blake and Hess, 2014; Ohanaka *et al.,* 2017). Plant ash from different sources have also been reported as a good source of mineral supplement in the diets of pullets (Okoli *et al*., 2014) and in growing rabbits (Iwu *et al*., 2013).

Incomplete combustion of the ARs under controlled oxygen environment will usually lead to activated charcoal or biochar production, which is a non-soluble powder with a high porosity, and surface area (Wang *et al.*, 2017; Schmidt, Hagemann, Draper and Kammann, 2019). It is believed to have enormous absorptive, and bacteriostatic properties in the gut and as such, it is capable of absorbing gases, anti-nutrients, bacteria, and mycotoxins present in livestock feeds, while supplying some essential minerals to the animals (Khoa, Quang, Thang, Phung, and Kien, 2018).

Investigations conducted on broiler chick's diet incorporated with dietary wood charcoal was shown to increase liveweight gain, feed/gain ratio, and feed consumption, while reducing odour, and domestic fly count in the faeces (Durunna, Abatai, Isaac, and Uchegbu, 2018).

Organic acids have also been used as additive in poultry feeds to prevent feed contamination from pathogens and its uptake from the gut, while improving the digestibility of nutrients (Khan and Iqbal, 2016; Dittoe, Ricke, and Kiess, 2018). For example, vinegar; a sour liquid synthesized from sugary substances through alcoholic fermentation, and as an organic acid can be added in poultry feeds to improve its quality (Allahdo *et al.*, 2018). It contains mainly acetic acid and other organic acids which possess bactericidal and performance enhancing properties (Khan and Igbal, 2016; Ahmed, Mun, Song, and Yang, 2018).

Tasharofi, Goharrizi, and Mohammadi, (2017) reported improved intestinal morphology, and performance in broilers when wood date vinegar (WDV) was added in their diets. A mixture of charcoal, and wood vinegar in poultry diets increased their feed intake, and weight gain (Samanya and Yamauchi, 2002; Rattanawut, 2014), while activating their absorptive functions (Samanya, and Yamauchi, 2001; Rattanawut, Todsadee, and Yamauchi, 2017). Such blends were reported to significantly increase hen-day production, and feed conversion ratio in laying hen (Yamauchi, Ruttanavut and Takenoyama, 2010; Rattanawut, Pimpa, Venkatachalam, and Yamauchi, 2021). Furthermore, these mixtures have been reported to elicit improved egg weight, eggshell strength, and thickness in laying birds (Rattanawut *et al.*, 2017; Elsahn, 2021).

Palm wine which is a natural fermented drink produced from the sap of tropical plants of the Palmae family such as *Raphia hookeri* and *Elaeis guineensis,* could serve as a ready source of organic acids, and microbial products when allowed to age beyond five days (Amoa-Awua, Sampson and Tano-Debrah, 2007; Nwaiwu *et al.*, 2016). It is a clear sweet syrup with high concentration of sugars, proteins, alcohol, organic acids, and microbial population (yeast, lactic acid forming bacteria and acetic acid forming bacteria) (Astudillo-Melgar, Ochoa-Leyva, Utrilla, and Huerta-Beristain, 2019; Djeni *et al.*, 2020).

Several reports have shown that the supplementation of yeast in poultry diets enhanced poultry performance through improved intestinal microbiota population, and digestive enzyme activity (Hana, Tyfor, Tabidi, Nasri and Mukhtar, 2015; Ahiwe *et al.,* 2020; Zhang *et al.,* 2020). Palm wine derived microbial products reportedly improved broiler growth when added in their diets (Bohoua, 2008; Alade, 2018). Thus, the yeast and organic acid content of aged-palm wine could be exploited to enhance the performance of poultry.

Therefore, a product made from AR-derived activated charcoal and aged-palm wine could produce physiological effects in broilers, and layers like birds fed a blend of activated charcoal, and wood vinegar (Rattanawut *et al.,* 2017; 2021; Sittiya, Yamauchi and Yamauchi, 2021). These effects may include increased liveweight gain, feed intake, egg laying rate, improved intestinal absorption of nutrient in layers as well as improved FCR in broilers.

1.2 Problem Statement

The problem of high cost of poultry feeds arising from high cost of feed raw materials requires an urgent solution to ensure the sustainability of the poultry industry in Nigeria. With the increasing cost of feed raw materials commonly used in commercial poultry rations, several alternative materials which are cheaper, and more readily available at specific locations have been suggested by researchers as possible solution to increasing feed cost. Attempts by the feed industry to formulate cost competitive diets from these alternatives have resulted in the production of low-quality commercial diets that led to low performance results when compared to recommended performance levels (Ukwu, Ogbuewu and Uchegbu, 2018). This has led to insidious production losses, especially among most small-scale intensive producers who depend on commercial diet brands for poultry feeding. Poor feed industry regulatory schemes, especially where there are no functional standards, compounds the problem for the farmers (Omede, 2010). Therefore, the adoption of feed additive inclusion in diet formulation becomes a viable option for farmers to optimize productive performance and reduced feeding cost.

Several commercial additives such as enzymes, amino acids, probiotic and organic acids are being used by large scale producers to enhance performance and productivity (Singh *et al.,* 2015; Arif *et al.,* 2020). While the use of additives has its benefits in such farms due to economics of scale of production, their use in small-scale farms is usually the opposite. It would therefore be advantageous to the resource-poor poultry farmers under small scale poultry farming conditions, if cheap and readily available feed additives could be identified for inclusion in the diets of chickens to upscale the utilization of available feed resources thereby promoting improved performance, health, and productivity.

Studies are increasingly reporting the performance benefits of activated charcoal (AC) supplementation in poultry diets. However, the practice is not yet popular because of limited information on appropriate materials to be used, production methods, and supplementation levels in poultry. It is well established though that functional activated charcoal could be produced from most agricultural residues such as pig and poultry dung (Lima and Marshal, 2007; Xiao *et al.,* 2018), which are the major outputs from livestock farming activities (Sillar, 2000). These animal dung that presently pose environmental challenges to the farms through odour emission and disposal difficulties could be converted into activated charcoal.

Most animal dung does not burn well, and therefore may be difficult to produce good biochar or activated charcoal from them (Iregbu, 2014). This constraint could be overcome by blending the dung with flame accelerants such as palm kernel shell and bamboo wood at appropriate levels. Though the performance effects derived from the use of AC in diets have been limited, some studies have however shown that a mixture of AC, and probiotics or AC, and organic acids potentiates/increases the performance results obtained from AC supplementation of poultry diets (Yamauchi *et al.*, 2010; Rattanawut *et al.,* 2021; Sittiya *et al.,* 2021). It is however not known if an activated charcoal produced from these blends, and or mixed with an aged palm wine, as dietary additives in broilers, and laying hen will produce such increases in performance results reported by these authors.

1.3 Objectives of the study

Aims: The aim is to determine the morpho-physiological responses, and performance of chicken fed activated charcoal – aged palm sap supplemented diets.

The specific objectives are:

i. to produce activated charcoal (AC) and activated charcoal – aged palm sap (ACAPS) feed additive for poultry feeding.
ii. to determine the effect of AC, and ACAPS supplementation on laying performance, egg quality characteristics, reproductive hormone, and tract morphometry, haematology, and serum chemistry in laying hen.
iii. to determine the effects of AC, and ACAPS supplementation on performance, carcass/organ characteristics, haematology, and serum biochemistry of the broilers.
iv. to determine the effect of AC, and ACAPS on mineral uptake, and excretion in faeces

1.4 Justification of the study

In Nigeria, agro-residues/wastes are in abundance. Farmers are the owners of these agro residues which so far are of limited economic value to them but rather a major contributor to environmental pollution with high nuisance value. Although pig dung has been used as organic fertilizer in crop farms, the increase in pig population in recent years has resulted in dung generation outpacing the demand as fertilizer (Ume, Ezeano, Onunka and Agu, 2018), and resulted in indiscriminate dumping, and environmental concerns (Oseghale, 2010). Therefore, any simple technology that will result in the reduction of dung volume, and mitigation of these environmental concerns will be beneficial to the pig industry in Nigeria.

The utilization of faecal waste beyond its traditional use as manure in cropping systems or as energy source for boiler operations, and as mineral sources in form of ash in poultry diet have been highlighted (Blake and Hess, 2014; Rashidi, Khatibjoo, Taherpour, Akbari-Gharaei, and Shirzadi, 2020). Bamboo charcoal and vinegar products derived from the pyrolytic combustion of bamboo have shown great promises as growth-promoting supplements in animal production (Chu *et al.,* 2013; Rattanawut *et al.*, 2017; 2019; 2021). Already studies conducted using ash derived from palm kernel shell suggest its potential use as a mineral supplement in broiler production (Ohanaka *et al*., 2017; Ohanaka, Okoro, Etuk, Unamba-Oparah and Okoli, 2018a; Ohanaka *et al.*, 2018b). Palm kernel shells are good precursor materials to produce granulated activated charcoal products employed in various industrial treatment operations (Ma *et al.*, 2017; Mohammad Razi, Al-Gheethi, Al-Qaini, and Yousef, 2018). Again, the blending of PKS and bamboo with the pig dung enhances the combustion characteristics of the pig dung. Therefore, the pyrolytic decomposition of such blends could yield ash/charcoal products that could serve as cheap adsorbents or mineral additives for dietary inclusion in poultry production, soil amendments/remediation or industrial treatment plants (Lima and Marshal 2007; Xiao *et al.*, 2018; Ohanaka, Ukonu, Ogbuewu, Etuk, and Okoli, 2021).

The development of a value-added product (AC and ACAPS) from a blend of these agro-wastes could serve as a practical waste -to-wealth initiative in small to large-scale farming operation. Such an initiative will not only produce a cheap feed additive but also increase access to feed additive by small-scale farms thereby increasing the productivity, efficiency, and income of small-holder farmers especially when utilizing alternative feedstuffs, and low-quality commercial feeds.

Activated charcoal have been employed as strong adsorbents in modern veterinary, and medical sciences (Jindal and Mahipal, 1999), and thus are able to neutralize anti-nutrients in ingested feed materials in the gut (Kutlu, Unsal and Gorgulu, 2001; Man, Chow, Man, Mo, and Wong, 2021). Toxins effectively bind to activated charcoal, which reduces its adverse effects on the gut flora (Prasai *et al.,* 2017; Schmidt *et al.,* 2019). Therefore, activated charcoal addition in on-farm feeds will promote digestion, and feed efficiency, while increasing energy gained from feed (Samanya and Yamauchi, 2002; Dim *et al.*, 2018; Kalus, Konkol, Korczyński, Koziel, and Opaliński, 2020).

The potential benefits of AC and ACAPS could also foster the growth of a new cottage industry that specializes in AC powder, briquettes, granules etc production through pyrolysis of agro-waste to generate detoxifiers/odour control products, thereby increasing the profits accruable from the sales of these products to farmers, and other individuals for other socio-economic benefits. The research findings from this study will also add information to science on activated charcoal and its application to poultry production. Such information is expected to drive policies on AR management, and other relevant information that will engender better environmental practices that promote waste minimization at farm and community levels, while instituting regulations, and sanctions for defaulters.

1.5 Scope of the study

In this research, pig dung, palm kernel shell and bamboo were used as precursors to produce activated charcoal (AC) using the physical method of activation. The AC product was further blended with aged- palm wine in a 7:3 ratio to decrease the dustiness of the charcoal powder without also significantly increasing the moisture content of the final product. The AC product and the treated diets were analysed for their physico-chemical characteristics. The effect of inclusion of these products at varying supplemental levels in experimental feeds were tested on layer, and broiler chicken to determine their influence on feed quality, growth performance, haematological, and serum chemistry, hormonal, and reproductive characteristics, faecal, and egg quality characteristic, carcass, and organ weight development.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Poultry Industry Situation in Nigeria

The agricultural sector is the largest domestic producer across the African continent and employs more than half of its total labour force (Outlook, 2013), and provides substantial household food requirements, and incomes. According to Heise, Crisan and Theuvsen (2015), agriculture has remained an important driver of economic activities in Africa, contributing about 30% of the national income, and large proportion of the overall exports. The sector remains the largest contributor of non-oil foreign exchange earnings to the Nigerian economy, providing employment opportunities to a significant population of her labour force (Phillip, Nkonya, Pender and Oni, 2009; World Bank, 2020). Specifically, the livestock sub-sector has shown promises towards the economic development of Nigeria. According to FAO (2019), around 13 million households or 40% of the total households in Nigeria keep farm animals which contributes 6 to 8 percent of the national GDP; through income generation, from food production, farm energy generation, and transport (Anon, 2006).

The poultry industry in Nigeria over the years has rapidly expanded to become one of the most capitalized subsectors of Nigerian agriculture with a chicken population of about 180 million birds, and second largest in Africa behind South Africa (SAHEL, 2015; FAO 2019). Of this total chicken population, about 40 million chicken or less than 25% are intensively farmed, while 60, and 80 million chickens are raised under semi-intensive, and extensive or backyard poultry systems respectively (FAO, 2019). According to the Central Bank of Nigeria (CBN), the poultry industry contributes about 25% of agricultural GDP with a current net worth that is valued at ₦1.6 trillion (The Cable, 2019). It has been estimated that a total of 650,000 metric tons of eggs, and 300,000 metric tons of poultry meat were produced by the industry in 2013

(FAO, 2017). A more recent report by FAO, (2019) estimated that about 454 billion tons of meat, and 3.8 million eggs are produced yearly in Nigeria.

Despite the promising outlook of the industry, Nigeria is far from meeting her domestic demands for poultry products (FAO, 2017). According to the CBN, the current demand situation for eggs, and meat are about 790,000, and 1,500,000 MT respectively which leaves a huge demand – supply deficit that unfortunately, is met through smuggling into Nigeria from Benin Republic (The Cable, 2019). This domestic shortfall of poultry meat was estimated at more than 1.2 million MT and will therefore require a chicken population of more than 200 million birds to meet the current domestic demand situation. The shortfall in production can be attributed to the excessive fragmentation of the industry, and with majority of its players producing under backyard/ extensive poultry system (Okeudo, 2004; Adene and Oguntade, 2006).

Smallholder farmers remain the major players, and providers of poultry products in Nigeria. This is in line with the recent survey reports of FAO (2019) which showed that the rural extensive poultry system has the highest number of industry players, with over 6.6 million or 83% of households involved in poultry production in Nigeria. Among these, 1.3 million or 16% of households are involved in the semi-intensive production system (68 million poultry) while only 1% (17000 commercial farms) intensively farmed 45 million poultry.

The backyard poultry production system is replete with outdated production techniques, equipment, inadequate hygiene management, and a weak feed industry (Adene and Oguntade, 2006; NEA, 2020). The astronomical rise in the cost of strategic inputs together with poor technical know-how characteristic of the production industry does not allow sustainable profit margins, which often threaten to force down farm operations (FAO, 2010).

Another important factor responsible for the supply deficit of poultry products in Nigeria is her growing population which has been projected to rise to 400 million people by 2050 with 280 million people living in the urban centres (UN, 2018; FAO, 2019). Increasing population dynamics, economic, and income scenarios have so far been identified as key factors driving the increased demand and consumption of animal protein, especially in developing countries (FAO, 2017; 2019). Nigerians are therefore expected to consume substantially more animal protein from poultry, beef, and milk, which have been projected to increase by 253, 117 and 577 percent, respectively by 2050 (Acosta and Felis Rota, 2016; FAO, 2019). However, these increases in demand, and consumption of animal protein will also drive an increased demand, and cost of inputs especially for the core components of animal feed such as maize and soybeans (Thornton, 2010), which further exacerbates the challenges already faced by the industry, and consequently will affect the productivity, and profit of technically poor poultry farmers (Okoli and Udedibie, 2017). To solve this issue, poultry production must be technically efficient, and the capacity of farmers (especially small holders) rapidly enhanced to meet up with the increasing demand (Olorunwa, 2018). This is possible as exemplified by the China model of enhancing the capacities of a multitude of smallholder farmers having an average of 5000 flock size of capacity per farm (Guyonnet, 2018) to become the largest egg producer in the world (FAOSTAT, 2020). The Nigerian poultry industry can also leap-frog if such example can be emulated.

2.2 Poultry Production Systems in Nigeria

The poultry production systems in Nigeria can generally be classified into two distinct groups viz: the commercial poultry production system and the backyard/rural poultry production system (Adene and Oguntade, 2008; NEA, 2020). The commercial production system is industry – oriented and favours the intensive farming of modern genetically improved poultry hybrids. It is highly capitalized and technically driven. Its production capacity ranges from a bird population of 100 - 5000 birds to fully integrated farms with (foreign franchise), high level of automation and stocking densities running in hundreds of thousands (Akandu, 2012; Heise *et al*., 2015). They have well established biosecurity, and disease control protocols which are strictly adhered to. While most integrated farms under this category import or manufacture their own drugs, and own feed processing plants to produce cheap and quality feeds for their birds, others depend on commercial feed millers, and veterinary shops for feed and medications.

On the other hand, the backyard poultry production system is associated with household or grassroot tenured chiefly characterized with non-uniform breeds, of chicken of various ages (Adene and Oguntade, 2006). The indigenous chicken forms an integral part of the rural chicken population which contributes a wide variability in the genetic resources of this group especially towards disease resistance, heat tolerance, meat hardiness and scavenging abilities (Nwogwugwu, Lee, Freedom and Lee, 2018). Unfortunately, most of these potentials are gradually lost with the production system especially when the best performing animals are mostly culled for the market (Odubote, 2015). A good example is the culling of the naked neck and frizzled feather chicken, that are better adapted to the tropical heat stress. The naked neck, and frizzled gene are known to confer improved feed conversion ratio, growth, meat, and egg production (Fathi, Galal, El-Safty, and Mahrous, 2013) to these birds when compared to their normal feather counterparts.

Bird population is generally on a small scale, extensively managed and subsistent in nature often with little or no veterinary inputs nor any form of biosecurity protocol (Pagani, Abimiku and Emeka-Okolie, 2008). Therefore, birds are burdened with diseases especially Newcastle disease virus, Gumboro disease virus etc. which account for over 60% of the mortality (Adene and Oguntade, 2006). Mechanization is practically non-existent with no climate control of the environment.

2.3 Constraints to Poultry Production in Nigeria

Despite the advancements made by the poultry industry in Nigeria in recent times, poultry production is still characterized by low productivity owing to a myriad of constraints bedevilling the efficient production and management of the enterprise among which include lack of credit, high costs of feeds, poor management, inadequate supply and poor quality of day-old chicks, diseases and treatment cost, and inadequate extension services (Alabi and Isah, 2002). Poor environmental conditions, poor quality feeds, and low inputs/outdated equipment among others have also been identified as other constraints driving the inefficiencies in the industry (Okoli *et al.*, 2005a).

2.3.1 Poor capital

Limited access to capital has been implicated as a critical factor responsible for low throughput of most poultry enterprises in Nigeria. The size of capital determines greatly the size of the operating units. Inadequate capital limits the capacity of farmers to acquire important technologies required for efficient and sustainable production (Esiobu, Onubuogu and Okoli, 2014; Olorunwa, 2018). Many farmers, especially at the subsistent level of operation, are unable to scale-up their operations because of low capital outlay, and the difficulties in accessing credit and, loan facilities required for the procurement of basic operational inputs. Therefore, the Nigerian governments as a matter of policy should relax the stringent conditions which make the process of agricultural loan procurement almost impossible (Aromolaran, Ademiluyi and Itebu, 2013; Esiobu *et al.*, 2014). This could be achieved through channelling available credits and grants to real cooperative association of farmers without huge collaterals.

2.3.2 Poor environmental conditions

The dry season in Nigeria is characterized by extreme heat, and dryness, while the rainy season is characterized by high humidity, and extreme wetness which often are harsh on chicken under production, hence the need for structures that minimize stress, while promoting ventilation, length of day, and general comfort. Heat stressed birds tend to consume less and drink more water with the consequence of lowered productivity, and immune system (Lara and Rostagno, 2013; del Barrio *et al.,* 2020). Increased water intake often translates to increased litter wetness/ moisture which breeds nuisance flies, and infectious diseases. Poor litter conditions may also lead to pollutant gases emissions, and accumulation in the poultry pen, which may again affect the health of birds, and human attendants (Nwagwu, Ede, Okoli, Chukwuka and Okoli, 2011). Observations have shown that poultry chicks are prone to diseases during weather extremes (Musa, Saidu and Abalaka, 2012; Abraham-Oyiguh *et al.*, 2014). Increased rainfall and relative humidity during the rainy season have been linked with the highest number of Coccidiosis, and Gumboro cases in Nigeria while, dry season with its hotness is associated with increased incidence of Newcastle disease, Gumboro, heat stress, and coccidiosis (Olanrewaju, Tilakasiri and Adeleke, 2015).

2.3.3 Poor quality and cost of feed

The availability of cheap, and qualitative poultry feeds is critical to sustainable poultry production (Mengesha, 2012). One of the major limitations of the poultry sector in Nigeria is the constant scarcity, and high cost of core feed materials. Feed cost has been implicated severally as the major contributor to high input cost in poultry production process (Nmadu, Ogidan and Omolehin, 2014; Esiobu *et al.,* 2014; Akintunde, Adeoti, Okoruwa and Abu, 2015) of which 70% of this cost goes to the procurement of energy and protein feed materials. Feeds, and raw material prices have continued to increase in Nigeria due to increased demand for food, and animal feed as well as the seasonal fluctuations in the production and supply (Olerede and Longe, 2000). The limited availability, and supply of major feed raw materials produced in Nigeria presents serious problems for local feed producers, who are often forced to reformulate poultry diets with low – quality protein, and energy sources (USDA, 2014). This affects performance and eventually leads to low productivity of the industry. Several researchers have reported that the nutrient composition values quoted on the label of some commercial feeds sold in Nigeria are misleading, and below declared values (Uchegbu *et al.*, 2009; Bukar and Saeed, 2015). According to Carew, Oluremi and Wambatda (2005), the energy (ME), and crude protein content of some major feed brands produced in Nigeria varied significantly from marginal to grossly inadequate, while crude fibre contents were far more than the recommended values. Alaoma (2016) reported higher moisture content, and crude protein (CP) contents below the recommended values for poultry in several commercial poultry feeds whereas, higher microbial counts were reported in some selected commercial poultry feeds sold in Nigeria (Okoli, Chah, Herbert, Ozoh and Udedibue, 2005b; Arotupin, Kayode and Awojobi, 2007). It therefore appears that commercial feed producers are making profit at the expense of quality, hence the call for quality control schemes that will ensure that standards in feed manufacturing are maintained (Okoli *et al.*, 2009).

2.3.4 Poor quality chicks' supply

Another major limitation to the growth of the poultry sector is the shortage of quality day old chicks (DOC). Given the expansion of the poultry industry in recent years, the demand for day old chicks has overshot its supply in most parts of the country. This situation coupled with the near absence of regulation or quality control of DOCs by the relevant authorities, makes a lot of hatchery operators to sell chicks of questionable pedigree or quality (Geidam, Bukar and Ambali, 2006), and at exorbitant prices. Again, such DOCs are often marketed characterized by high brooding period casualty and chronic stunting, which when sold to unsuspecting farmers, results in huge economic losses. Esiobu *et al.* (2014) had identified poor chick quality as a factor that can negatively impact investment capital in the poultry industry. The government regulators should therefore design and enforce better control of chicks' distribution in Nigeria and make sure that only quality stocks are sold to farmers.

2.3.5 Diseases and health care problems in poultry production

Poultry diseases are conditions that afflict poultry thereby affecting their productivity, wellness, morbidity, and mortality when not treated. Diseases are major constraints to livestock improvement with negative effects on productivity, production cost, and derivable income (Sadiq and Mohammed, 2017). Poultry diseases like Newcastle disease, coccidiosis, Gumboro disease, salmonellosis, fowl pox, avian influenza, and worm infestation are known for their significant effect on poultry production (Usman and Diarra, 2008). Newcastle disease is however recognized as the most important disease plaguing poultry in Nigeria (Fadiga, Jost and Ihedioha, 2013; Shittu, Joannis, Odaibo and Olaleye, 2016). Viral infectious diseases and coccidiosis have also caused substantial revenue losses to poultry farmers (Mshelia *et al.,* 2016) through reductions in feed intake and efficiency, immunosuppression, increased morbidity, mortality, and low-quality products.

The estimated economic burden of disease on livestock production in Nigeria has been put at about 29.2 billion Naira per year, with losses from Newcastle disease outbreak alone amounting to 8.9 billion Naira (Fadiga *et al.,* 2013). Significant economic, and job losses in the poultry industry were associated with viral diseases particularly Newcastle disease, Gumboro disease and bird flu (Muteia, Oparinde and Maina, 2011; Sadiq and Mohammed, 2017). According to Musa *et al.* (2012), farmers incurred losses to the tune of 3 billion Naira because of Gumboro disease outbreaks over the period from 2009-2011, while some farms in Abuja alone recorded losses ranging from 3.5 -5.2 million Naira through mortalities, and culled birds resulting from Newcastle disease outbreaks (Sadiq and Mohammed, 2017).

The outbreak of Avian influenza in Nigeria between 2006, and 2008 affected about 3057 farmers and about 1.3 million birds with the consequential job losses for which the Nigerian government paid 900 million Naira in compensation (FDL, 2008). The costs of medication, and vaccination against disease outbreaks also contributes substantially to the overall cost of production (Esiobu *et al.*, 2014). The high cost of disease management may result in the farmer being unable to budget the required amount to disease treatments, and prevention. Proper medication, vaccination, and general biosecurity measures are needed to ensure optimal productive performance in poultry farms.

2.3.6 Training and extension service problems in the poultry industry

The information at the farmers disposal on important husbandry practices, biosecurity standards, and market/marketing, are usually lacking because of the collapse of government extension services in the country (Nmadu *et al.*, 2014). While it has been shown that adoption of recent technologies in poultry production can bring substantial improvements in the productivity, through reduction of inefficiency (Aboki, Jongur and Onu, 2013; Esiobu *et al.*, 2014), the adoption of these innovations among most poultry producers in Nigeria remains low due to the limited delivery of extension services. Extension contacts are known channels through which recent agro – innovations, and information are disseminated to farmers for improved production (Onubuogu, Esiobu, Nwosu and Okereke, 2014). Similarly, poor government policies, and lack of infrastructures inimical to agriculture development, make farmers to continue in their trial and error with the resultant outcome often of failure (Heise *et al.*, 2015).

2.4 Feeding Constraints in Poultry Production

The availability of quality feed plays a critical role in the poultry industry. Properly fed animals are more resistant to diseases and produce well. Therefore, for maximum efficiency in production and management, adequate, and well-balanced poultry rations must always be provided. Poultry feeding in Nigeria has however been constrained by two major factors, which are high cost and poor quality of feedstuffs and finished feeds.

2.4.1 High cost of feed materials and finished feeds

The growth, and productivity of the poultry industry in Nigeria has been majorly constrained by the exorbitant cost of finished feed, and feed raw materials, which constitute about 70-80% of the total cost of poultry production (Nmadu *et al.,* 2014). The high cost of feed is directly linked to the high cost of feed ingredients that constitute the core energy and protein sources in monogastric animal feed production, and therefore limits the opportunities, and advantages of poultry production in Nigeria. According to Kekeocha (1998), energy source in the form of maize, constitutes about 50 - 70% of the total feed mixture, while protein, and vitamins/minerals sources constitute about 10 – 20% and 5% respectively. The ever-growing demand for important feed grains particularly maize, and soyabean for food, industrial and livestock uses has resulted in their continued price increase, hence the need to consider looking for worthy alternatives (Mengesha, 2012; Tona, 2018). The supply - deficit situation of feed raw materials may continue to worsen with the increased diminution of land areas for crop cultivation due to urbanization, population growth, climatic factors (drought and flooding), and more recently the insurgency situation (Boko Haram and Fulani herdsmen attack) in the savannah regions (supports grain production) in Nigeria. Again, the increased cost of other variables such as additives, vaccines, labour, and equipment, required for production due to inflationary trends altogether negatively affects the revenue accruable from poultry production

(Yusuf, Tiaminu and Aliu, 2016).

2.4.2 Poor quality of feed raw materials and commercial feeds

The quality and safety of feed raw materials ensures the production of quality, and safe animal food products (Tona, 2018). According to Okoli, Uchegbu, Ogbuewu and Omede (2009), feed quality is not just the nutritional/chemical characteristics of a feed/ ingredient but also the microbiological, pharmacological, and physical characteristics of feed or feed raw materials as well as an established legal scheme for quality assurance. Until recently, the physical evaluation of feed was done mostly to provide preliminary information on the quality of the material used in formulating the diet. This involves assessing qualities such as weight, colour, smell and whether the material has been contaminated by other materials (Okoli *et al*., 2009). Chemical evaluation assesses the nutrient composition, and toxicological status of the feeds.

In many developing countries, monitoring feed quality is mostly limited to proximate evaluation to reveal only the biochemical quality of feeds and feedstuffs. This gives a limited representation of the feed quality considering that many feedstuffs included in feed formulation, if properly evaluated, may reveal answers to many questions on low performance by animals (Adene and Oguntade, 2006). For example, even with apparent adequate rate of feed supply, animals have been observed to perform poorly in terms of growth, and other production parameters (Ukwu, 2013). This has been attributed to insidious environmental factors, lower feed ingredients quality, and a wide variability in nutrient composition (Omede, 2010; Okata, 2016) may be major contributing factors. Therefore, beyond the chemical composition of feed ingredients, the extent of utilization of these components by the bird, termed nutritive value, should also be measured. According to Omede (2008), feed quality could be assessed in terms of nutritional, physical, microbial, and toxicological quality, while quality schemes should be established to serve as the regulatory framework for the industry.

a) Nutritional quality: The nutritive value of a poultry feed is a function of its chemical composition, and the degree to which it can be digested, absorbed, and utilized (Wiseman and Inborr, 1990). Several researchers have consistently reported that the nutrient composition quoted on the label of some commercial feeds sold in Nigeria do not reflect their actual nutrient content and therefore misleading (Okoli *et al.,* 2007; Uchegbu *et al.,* 2009; Bukar and Saeed, 2015). Alaoma (2016) also reported higher moisture content, and low CP below the recommended values for poultry in several commercial poultry feeds sold in Nigeria. According to Carew *et al.* (2005), the energy (ME) and protein concentration of the major feed brands sold in Nigeria varied significantly from marginal to grossly inadequate, while crude fibre contents were more than the recommended values. Limcangco-lopez (1987) cited in Omede, (2010) reported that marked variations in CP content of feeds may be accompanied by changes in amino acids content, and that most developing countries do not have established ME values for feed ingredients. If the labels carried true estimates of nutrients, end-users of the feeds may be able to take steps to correct the deficient/limiting nutrients.

The problem of nutrient variation in feeds is further compounded using alternative or novel feed materials that lack reliable estimates of their nutrient contents, and levels of inclusion adjudged safe for various types of poultry (FAO, 2011). According to Adeola and Olukosi (2009), in order to effectively utilize alternative feed raw materials, there will be the need to properly understand what they are, how they can be processed for use as feedstuff, and what processing techniques will make them amenable to utilization.

Again, the use of formulation tables of materials assayed with different processing methods a very long time ago in today’s production might give false estimates of the feed values. In many situations, commercial feeds produced are consumed by animals before any assay could be performed. However, finished feeds assays should be mandatory (Jones, 2005). Limits of inclusion also should be properly established and critically controlled to determine if nutritive additives, and nutrient enhancers should be added in feeds before, and during production (Ukwu, 2019).

b) Physical quality: The physical characteristics of any feed or feedstuff is a term that includes all the quantitative, and qualitative expressions of the material features of such feed/feedstuff that have visible, and tangible attributes and that can be used in describing its natural structure (Omede, Okoli and Uchegbu, 2011). All feed ingredients are usually inspected for abnormalities of colour, mouldiness, off odour, fibrous nature, dustiness, and other factors that may result in quality risks when added to the feed. Over the years, poultry farmers have allegedly accused some greedy feed raw material suppliers of knowingly adding off- materials such as sand, saw dust, ground maize cobb in maize etc. in the various raw materials meant for animal feed production, thereby reducing their nutritive quality, while increasing its susceptibility to weevils and mould growth (Omede, 2010).

However, four important physical characteristics that greatly influence the nutritional value of poultry diets are feed bulk density, water holding capacity, particle size, and specific gravity (Sundu, Kumar and Dingle, 2005, Okoli, *et al*., 2009; Omede, 2010). Others may include dry matter digestibility (De Lange, 2000), and moisture content. Therefore, neglecting the evaluation of physical quality characteristics of poultry feeds might be one hidden reason why some intensively kept animals eat below their productive requirements in the tropics (Ukwu *et al*., 2018).

c) Microbial/ microbiological quality: Poultry feed resources are commonly contaminated with micro-organisms, mostly bacteria, and fungi. The growth of microbes on feed materials is dependent on climatic conditions for harvesting, processing, storage, and transportation system as well as packaging materials employed (D’mello, 2006).

Results of studies conducted by Okoli *et al*. (2005b), and Arotupin *et al*. (2007) on some selected commercial poultry feeds sold in Nigeria showed that microbial counts were much higher in the broiler feeds than the layer, and grower feeds, while the layer mash had higher fungi contamination than the other feeds. This reflects the contamination picture of ingredients especially the animal protein, and oil seed sources used in producing the rations (Okoli *et al.,* 2005b; Okoli, Ekwuagana and Ogbuewu, 2006). Again, broiler diets are high energy diets which often require oil seeds, and animal protein to shore up its energy content (Esonu, 2015). This practice may aid the rapid multiplication of fungal organisms in the feed.

Therefore, the microbiological criteria for establishing quality in feeds must be clearly outlined, and strictly adhered to. This is imperative in view of the frequent poultry infections, and disease outbreaks across Africa, and tropical countries that leave trails of destruction, and huge financial losses in farms. This will also reduce the transfer of zoonotic organisms, especially *Salmonella,* and *Escherichia coli* to poultry farm operators. Van Immerseel, Cauwerts, Devriese, Haesebrouck and Ducatelle (2002) reported that leading causes of human food borne infections in the world such as *Salmonella* and *Camphylobacter* are associated with consumption of poultry products. Therefore, important organisms such as *Salmonella*, moulds, and mycotoxin producing fungi need to be tested for, before any feed can be declared safe for animal consumption (Okoli *et al*., 2006a, b; Okoli, Nweke, Okoli, and Opara, 2006).

d) Toxicological quality: Formulating poultry diets with low quality non-conventional feed resources as ingredients may reduce the quality of the finished feed. Some of these ingredients are known to contain some level of toxins and anti-nutrients even after processing which can vitiate the overall productivity of the birds (Adeola and Olukosi, 2009). Anti-nutrients are those substances present in plant materials that impact nutrient utilization in animals (D’Mello, 2000).

Mycotoxins are poisonous chemical substances or secondary metabolites such as aflatoxin, vomitoxin, zearalenone, ochratoxin, and trichothecenes produced by actively growing moulds. They are known to elicit negative performance responses in poultry; hence the need to regulate their presence or absence in feeds, and feed ingredients (Ugwu *et al*., 2017). Regulations for mycotoxins in feeds other than aflatoxins hardly exist in Africa (Kpodo and Bankole, 2008; Siame and Nawa, 2008) due to the near lack of information on the micro-flora and mycotoxins contamination levels of feedstuff (Okoli, 2005; Okoli *et al.,* 2006c). There is also a lack of awareness by regulators due to limited research attention dedicated to mycotoxin monitoring, especially among animal scientists (Okoli, 2005). However, feed industry regulators all over the world are very concerned about the presence of mycotoxins in animal feed, and animal food products. Hence, there is a need for the Nigerian feed industry and other stakeholders to set up post-harvest research platforms aimed at developing methods for monitoring and combating mycotoxins contamination in grains destined for animal feeding (Okoli, 2005b; Njoagwuani and Okoli, 2017).

Again, feeds must be tested for other toxic environmental contaminants such as pesticides, and residual toxic levels of certain metals or accidental additions during processing. Mercury poisoning and accumulation have been the most frequently associated contaminants of animals fed grains treated with mercury-containing dressing to prevent fungal growth (Aladi, 2003), while pesticides contamination may arise from activities in the field, and during post-harvest storage (Njoagwuani, 2017).

e) Quality schemes and regulations: Animal Feeds are produced mainly to optimize the genetic potentials of the targeted animal. Essentially, the quality, and safety of feed and feed ingredients should also be prioritized. This is because, feed ingredient quality, and safety are critical to the production of safe, and quality feed, and quality animal food products (Sharma, Sharma and Datt, 2015; Malomo and Ihegwuagu, 2017). The safety of animal feed ingredients should specifically be assessed prior to their use in diet formulation, and feeding (Njoagwuani, 2017).

In developing countries like Nigeria, the agencies saddled with the responsibility of quality control in the feed industry are yet to fully come to terms with the challenges of producing safe, and quality feeds. While there is some level of regulation of poultry feeds, the regulation of feeds for pigs, and other livestock is lacking. There is hardly the existence of relevant laws that will compel feed manufacturers to evaluate, and regulate feed quality (declare pharmacological, microbiological, and toxicological status). This, according to Njoagwuani (2017), is chiefly because the laboratory facilities to support sanctions when needed are inadequate and expansion is limited by competing needs. While Nigeria has a Feed Safety Management Committee that is fully conversant with the CODEX guidelines and WTO's SPS compliance to trade in agriculture, including animal feed, there is limited capacity to implement the desired objectives.

In the absence of functional relevant laws therefore, mixed feed is manufactured, distributed, and sold in Nigeria with limited quality control. This is because, the feed quality standard for poultry feeds in Nigeria as specified by the Standards Organization of Nigeria (SON, 2018), focuses on the nutrient requirement or levels for different types of poultry, while critical issues relating to specific types and levels of inclusions of feed additives, and medicants, allowable pathogenic microbes and toxicant levels, especially mycotoxins, and heavy metals, and their inhibitors are overlooked (Okoli *et al*., 2009; Omede, Okoro, Uchegbu, Okoli, and Anyanwu, 2012). The document is also silent on process control and personnel monitoring of procedures.

There should be provisions of essential laws to protect feed consumers to some extent since animal feeds are increasingly being incriminated as a health risk for different operators along the production chain (Ugwu, Unamba-Opara and Okoli, 2017). Although there may be quality control laboratories, there is no mandatory government – imposed quality control culture presently in Nigeria and it is left mainly to the feed industry to monitor itself (Njoagwuani, 2017). Like other industrial manufacturers, feed manufacturers should be required to register their manufacturing, distributing, and sales operations (Omede, 2008). They may have their finished feeds analysed to obtain certification from the Standards Organization of Nigeria (SON). Laboratory personnel, mill crew, the office staff, the nutritionists, and several other persons involved in feed manufacturing must come under such laws. Imported foreign feed ingredients or finished feeds must also be licensed by SON or any other appropriate authority.

2.5 Approaches to Reducing Feed Cost in Nigeria

The growth and sustainability of the poultry industry in Nigeria has been greatly constrained by the increasing cost of feeds and feedstuffs due to supply – deficit of core feed materials. In the face of this competitive raw material demand, and the increasing human population, researchers have suggested the adoption of non-conventional feed resources (NCFR) not directly utilized by man as feed ingredient in poultry nutrition to solve the problem (Esonu, 2009). Such inclusions help to reduce the cost of feed procurement, and minimize direct competition between man, and the livestock industry for available food grains. Other approaches that have also received some research attentions are techniques for manipulating feed form and feeding methods. Feed additives application to aid various aspects of feed utilization and feeding values have also become common practice.

2.5.1 Search for cheaper readily available alternative feed raw materials

Alternative feed resources or NCFRs are generally feed resources that have not been traditionally used for feeding livestock or are not normally used in conventional livestock diets in a given location (Amata, 2014; Ifeduba, 2018). They are usually the by-products of agro-industrial processing of plant, and animal products (Etuk *et al*., 2013; Uchegbu, Okata, Omede and Okoli, 2017). The advocacy for NCFRs stems from their local availability, relative low cost, ease of processing and rich nutritional content. They have been reported in some cases to be rich sources of nutrients (Olomu, 2011; Ukonu *et al.,* 2018). For example, bye-products from local agro-processing such as the brans, middling and husks of cereals, peels of cassava, plantain, and yam peels, among others, have been suggested as rich sources of nutrients for poultry feeds (Uchegbu *et al.,* 2017).

Yam peel for example has been reported to contain a substantial level of energy, and crude protein value of 2604 kcal/kg and 11 %, respectively (Amusa, Kehinde and Ashaye, 2002) capable of significantly reducing the energy cost in broiler diets, with no deleterious effect on performance (Ukonu *et al*., 2018). This is in line with the submissions of Etuk *et al*. (2013) who reported reduced cost of feed per kilogram of weight gain, when a combination of plantain peel meal (PPM), (3334 kcal/kg metabolizable energy) yam peel meal (YPM) and palm kernel cake (PKC) partially replace maize as an energy source in starter broiler diets. Similarly, cassava peel meal has been shown to substantially replace maize as an energy source in both broilers, and layer production (Adeyemo and Sani, 2013; Mbachu *et al.,* 2018). Raw wild cocoyam corms when added in finisher broiler diets have also been reported to significantly reduce the cost per feed intake in broilers even at higher inclusion levels (Olajide, 2012).

Interestingly, insects and worms are being researched as cheaper protein source, with about 40–75% CP content, and therefore capable of replacing costly conventional oil seed proteins (Kareem *et al.,* 2018; Alshelmani, Abdalla, Kaka, and Basit, 2021). Specifically, larvae of insects and worms such as maggots, earthworms, and termites have been found not to compromise performance in poultry when incorporated in poultry diets (Okah and Onwujiariri, 2011; Khan, 2018).

Other known examples of ingredients that have been researched as possible alternatives to conventional feedstuff in poultry diets in developing countries include bakery, and confectionary by-products (Helkar, Sahoo and Patil, 2016), fruits and fruits by-products such as Molasses, sugar cane scrapings, and citrus offals etc. (Ajuonuma and Awodi, 2012; Alu, Kaankuka and Kapechi, 2012), and leafy meals (Ifeduba, 2018).

These ingredients can be manipulated to provide the energy and protein needs of poultry at reduced cost. To this end, ingredients are often priced favourably enough to be used at large to replace all or part of the more common energy sources (maize and wheat). Similarly, by-products of meat processing such as broiler offal or hydrolysed feather meal can efficiently replace fishmeal in poultry diets due to their richness in quality protein, and essential amino acids, thereby forcing down the cost of production (Haryianto, Purwaningrum, Andityas, and Wijayanti, 2017; Naveed, Sharif, and Sultan, 2019). These less common or novel ingredients are however not without their problems, and as such, care must be taken before inclusion in diets in excessive amounts (Adeola and Olukosi, 2009). This is due chiefly to their contents of high fibre, limited available nutrients, anti-nutritional properties, and low digestibility, which limits performance, and productivity in birds (Katoch *et al.,* 2018). Again, the near absence or lack of standardized data on their nutritive value (Okata, 2016), and safe levels of inclusion in poultry feed (FAO, 2011) also limit their utilization in poultry rations.

Therefore, studies are required to ascertain their economic feasibility under practical farm conditions. However, it has been suggested that with proper processing, and treatment using functional additives; some of these novel feedstuffs such as agricultural waste/residues could be better utilized as feed ingredients in poultry production (Katoch *et al.,* 2018; Bhuyan *et al.,* 2020), especially through the reduction of their anti-nutrients and metabolites below threshold levels (Olomu, 2011; Alshelmani *et al.,* 2021).

2.5.2 Manipulation of feed forms

The physical form of a feed has a significant impact on the performance of chicken (Agah and Norollahi, 2008). Poultry feeds in mash form are finely ground such that birds cannot easily separate-out different ingredients during feeding, while pelleting is the act of modifying mash feeds into pellets through mechanical extrusion. Though the manipulation of feed form could increase the initial cost of feed, however, the improved performance of poultry because of such manipulations cancels the cost incurred because of such processing (Esonu, 2015).

The feeding of pellets to broilers improves growth performance due to increased intake of feed, higher feed digestibility, decreased ingredients segregation, and selective feeding, reduced microbial burden in feeds, reduction of energy expended in prehension, and increased palatability (Abdollahi, Ravindran and Svihus, 2013; Lv *et al.*, 2015). This reduces feed wastage and decreases selection of preferred individual ingredients by the birds compared to mash diets (Gadzirayi, Mutandwa, Chihiya, and Mlambo, 2006).

Crumbling of feed is another form of feed modification which involves the crushing of already pelleted feed to a consistency coarser than mash. Proudfoot, Hulan and Mc Rae (1982), reported that birds fed a crumble-pellet diet grew more rapidly and this has made this form of feed to become popular in broiler production due to its convenience of feeding.

Particle size (PS) of feedstuff have great importance in regulating feed consumption due to its association with feed intake and development of the digestive organs of young birds (Iwuji *et al*., 2013). Although the reduction in the particle size of feed for poultry may improve the handling and mixing of the ingredients during production while providing greater access of the feeds to digestive enzymes (Omede, 2010), however, it may also present its own challenges as birds may encounter difficulties consuming very fine or coarse particle feed (Omede, *et al*., 2011; Iwuji *et al.*, 2013). Coarse particle size feeds are more retained in the GIT and reduces water excretion in droppings through increased water reabsorption from the GIT compared to fine particles (Syafwan, Kwakkel and Verstegen, 2011). Recent study by Kheravii, Swick, Choct, and Wu (2018), also reported that coarse feed could upregulate genes expressions for important digestive enzymes, and nutrient transporters in broilers. However, it is important to note that young birds cannot eat very large particle sizes of feed, as their beak is still small. When particle size is larger than beak size, it results in reduced weight gain, and feed intake during the rearing phase (Frikha *et al.,* 2011).

Bulk density (BD) is expressed as weight of dry ingredient per unit volume (g/cm3). It has been reported as one of the factors affecting the acceptability of feed, and storage volume, particularly in the digestive tract of young poultry chicks (Sundu *et al*., 2005). Studies on BD by Shelton *et al.* (2005), tend to suggest that low BD diets significantly lowered the body weight of growing chicks due to decreased energy volume ratio of the diets. Studies by Omede *et al*. (2011), indicated that the metabolizable energy intake of birds was improved when dietary BD was increased, and that BD affected the growth of younger birds more than those of older birds. According to the author, the limited gut capacity of young birds to hold digesta limits the amount of feed and energy consumed. As the birds get older, the capacity of the gut to hold food, and possibly to break down non starch polysaccharides (NSPs) increases due to gut development, and therefore the birds become more tolerant to low BD diets. Therefore, when feeding poultry chicks, the BD properties of the diet formulated must be considered so as not to hinder intake and growth.

2.5.3 Feeding methods

Feeding systems that reduce cost of feed can be achieved through the method and period the chicken is offered the diet. The feeding of birds in the early morning hours before sun rise, and during cool evenings ensures that no nutrient or energy is lost to the environment in the form of heat increment (HI), especially under hot tropical environments (Syafwan *et al.*, 2011;

Nawab *et al.,* 2018). This is called regimental or gap feeding. Briefly, the birds are fed their normal requirement and allowance 2 or more times a day with a 3-4-hour gap without feed. This method of feed restriction also allows birds to consume fine particles, thereby improving feed-gain ratio, and body weight uniformity during production (Hyline International, 2015), and at the same time reduce feed wastage.

The wetting of poultry feed with water is another management practice that improves performance, and digestive efficiency particularly in heat-stressed poultry (Syafwan *et al.,* 2011; Saeed *et al.,* 2019). It is believed that such improvement was because of increased feed consumption, improved digestion/absorption, and reduced viscosity of gut digesta compared to dry feeds (Yasar and Forbes, 2000; Lin, Jiao, Buyse, and Decuypere, 2006).

Sequential feeding has been described to involve the feeding of different energy, and protein levels in the feed offered in the morning versus the afternoon feed (Traineau *et al.,* 2015). The reduction of feed wastages will reduce cost of feed. Feed wastage could be controlled using efficient feeding trough/ chain feeding systems, adjusting feed levels in the feeder (avoid over filling), controlling feed spoilage while checkmating rodents in the farm during production.

Studies have also shown that chicken can discriminate between coloured feeds, and feeders and will alter consumption in response to the different colours (Kruger, 2008; Lecuelle *et al.*, 2011). For example, newly hatched chicks will prefer a green-coloured material as a potential source of feed over red coloured one (Ferket and Gernat, 2006), whereas Hurnik *et al.* (1971) reported highest feed intake from red coloured feeders. Again, yellow, and red coloured feeders were more preferred by broilers than blue, and green and black (Weeks *et al.,* 1997), while turkeys had highest feed consumption from green coloured feed troughs (Cooper *et al.,* 1971). Effect of feeder heights on intake and performance has been assessed (Neves, Banhazi and Nääs, 2014). When feeders are regulated too high, feed consumption by smaller birds are likely to be inhibited, thereby resulting to uneven growth of the flock (Ferket and Gernat, 2006). On the other hand, Quintana *et al.* (1998) reported increased body weight gain at lower feeder heights, with a lower percentage fat deposition in the thighs and greater muscle in drumsticks (Roll *et al.*, 2010). The higher adjustment of feeder heights for laying birds has a positive influence on hen posture and survival, thus reducing incidence of feather pecking and cannibalism (Freire, Walker and Nicole, 1999).

Daylength or lightening duration influences feed consumption, and performance. Slight increase in feed consumption has been observed in birds at dawn, and shortly before dusk, especially when natural lighting program is being used (Kruger, 2008). However, increasing daylength during production by using additional lighting programs will not only increase feed intake and growth performance, but will decrease age at sexual maturity for growing pullets, and improve egg production through increased ovarian functions (Jacome, Rossi and Borille, 2014; Patel, Patel, Patel, and Patel, 2016).

2.5.4 The use of feed additives to improve poultry performance

Feed additives are substances, that are intentionally added to animal feed to improve productive performances of animals. According to Singh, Chandramoni, Kumar, and Kumar (2015), such substances when present in feeds modify its characteristics, intake, digestibility, gastro-intestinal flora of the animal fed, and the quality of the final animal product. Their utilization in commercial animal production has been very extensive over the years. They are mostly added to feeds in small quantities as defined by law and as prescribed for different categories, and ages of animals.

In intensively farmed poultry, additives are specifically added into feeds to improve intake, digestion, absorption and/or metabolism efficiency, and/or the health of birds (Watts, Lawrence, and Lawrence, 2013). Common examples of routinely used additives include enzymes, antibiotics, prebiotics, probiotics, acidifiers, antioxidants, vitamins, minerals, amino acids, and binders, which when applied in feeds, evoke performance responses in animals through improved efficiency in nutrient uptake and utilization (Singh *et al.*, 2015; Abd El-Hack *et al.,* 2020). The benefits of additives supplementation in diets could also be divided into three main categories viz; additives that improve the quality of poultry feeds, additives that improve feed intake, and additives that improve feed nutrient utilization and uptake.

2.6 Categories of Feed Additives used in Poultry Production

2.6.1 Additives that improve the quality of poultry feeds

Additives could be produced and added to poultry feeds with the aim of enhancing its physic-chemical characteristics or preserving the quality of the feed already produced. Certain additives have been suggested to improve or maintain the quality of poultry feeds.

(a) **Mould inhibitors/Toxin binders:** Mould inhibitors and toxin binders are additives added in poultry feeds to prevent the growth of moulds already present in feed raw materials or finished feeds during handling, processing and/or storage, or during unfavourable weather conditions (D’Mello and MacDonald, 1997). Most cereals and oil seeds commonly used in diet formulation are prone to mould growth. Moulds have been implicated as the chief culprit responsible for mycotoxins contamination in feeds which are poisonous secondary metabolites produced by actively growing moulds (Njoagwuani and Okoli, 2017). Mycotoxins such as aflatoxin, vomitoxin, zearalenone, ochratoxin, and trichothecenes when present in feeds are known to elicit negative performance responses in poultry when consumed (D’Mello and McDonald, 1997).

One of the commonest mould inhibitors is the organic acid (propionic acid) which has been found to inhibit mould growth in feeds (Khan and Iqbal, 2016). However, it is not so much effective against mycotoxins produced by these moulds. Therefore, mycotoxin binders are added to prevent toxin uptake from the gut and into the bloodstream (Opara and Okoli, 2005). Mycotoxin binders include but not limited to clays products (bentonites and zeolites), yeast cell derivatives, activated charcoal, alumino – silicate preparations etc. (Njoagwuani and Okoli, 2017).

(b) **Antioxidants:** Antioxidants are additives utilized in the poultry industry to enhance the quality of feeds, and animal products. They are specially recommended for diets containing high concentrations of fats. High fat containing ingredients such as fish meal, full fat soya, groundnut cake etc., are often added to poultry diets to increase their energy contents, especially in high density broiler diets. Unfortunately, such ingredients have the tendency to go rancid when included in high proportions in formulated poultry diets stored for a prolonged period at higher temperature conditions (Ezeokeke, Okpogode, Okoli, and Esonu, 2008). Rancidity is an oxidative reaction that occurs in high fat diets due to unfavourable storage conditions or feeds containing free radicals (Esonu, 2015). The inclusion of antioxidants to feed is an effective way of stopping feed spoilage by binding the free radical molecules known to cause deterioration of feeds. Common examples of antioxidants include vitamin C and vitamin E, butylated hydroxytoluene (BHT), butylhydroxyanisole (BHA), and ethoxyquin, (Singh *et al.*, 2015; Wortinger and Burns, 2015).

(c) **Additives that improve texture and appeal:** Feed additives may be supplemented in poultry feeds to improve the properties of the feeds or to improve consumer acceptance of a product. Additives such as the pellet binders, flow enhancers, colouring and flavouring agents fit into this category.

Pelleting of feeds has been shown to improve feed efficiency for some poultry species, especially broilers (Amerah, Ravindran, Lentle, and Thomas, 2007). Free-flowing additives are added to feeds prior to pelleting to help improve the quality, and durability of the pellets to reduce feed dust and clumping of feed (Ugwu and Okoli, 2017). An example of a flowing agent is hydrated sodium calcium alumino – silicate due to its ability to prevent caking of feed during, and after production (Grenier and Applegate, 2013). Feed materials such as molasses, clays, starches and various lignin and hemi – cellulose products are common examples of pellet binding agents that can improve pellet quality.

Colorants like yellow, orange, red and green are often included in feed to mask changes in ingredient composition, while increasing feed acceptance/preference by chicks, and poults (Weeks *et al.,* 1997; Lecuelle *et al.,* 2011). Again, natural colour-producing bioactive extracts from plants such as xanthophylls, carotenoids, capsanthin, capsaicin, zeaxanthin, lutein etc. added to poultry diets, have been shown to influence the coloration of egg yolk or broiler skin (Lokaewmanee *et al*., 2013; Arimboor *et al.,* 2015). Such colorations in animal products influence consumer appeal through sensory evaluation of foods (Hamelin and Altemueller, 2012). For example, a well-pigmented egg yolk will appeal more to consumers than if otherwise.

Flavour and aroma stimulating agents (citric and phosphoric acids) are frequently used to improve palatability of feed ingredients that may otherwise have bitter taste, thereby enhancing feed consumption. It can also keep birds on feed especially during disease or stress conditions, thereby helping to enhance feed intake (Damron, 2008).

2.6.2 Feed additives that enhance feed intake

While pellet additives and flavour enhancers can be said to enhance the palatability and feed intake in poultry, certain other feed additives such as electrolytes, appetite stimulators, and yeast among others have been reported to improve feed intake in poultry. Additives such as enzymes, antimicrobials, probiotics, prebiotics can positively influence feed consumption, and nutrient utilization in livestock and poultry (Upadhayay and Vishwa, 2014).

(a) Electrolytes: These are products, which when dissolved, dissociate into positive and negative ions in a suitable medium and have an intrinsic ability to conduct electric current. The ionic concentrations of sodium (Na^+), potassium (K^+), and chloride (Cl^-) are the leading elements involved in electrolyte exchanges because of their high bioavailability, and relatively higher proportion in the body (Hooge, 2003). These electrolytes are critical for intra and extracellular homeostasis and influence variety of biological parameters within the body ranging from acid – base balance, osmotic pressure, impulse transmission, enzymatic activity, and provision of platform for optimum performance in poultry (Ahmad and Sarwar, 2006; Borges, Da Silva and Maiorka, 2007). They are also critical in nutrient utilization, bone development, and growth, (NRC, 1994), especially in poultry reared under tropical climatic environments and heat stress.

Heat stress forces birds to pant in order to reduce their body temperature, leading to loss of carbon dioxide, bicarbonate ions, sodium and potassium ions from the body (Da Silva, Flemming and Franco, 1994), therefore causing respiratory alkalosis. These metabolic alterations will often cause reductions in feed consumption, growth, liveability, and in turn profitability (Deaton *et al*., 1986). Yalcin *et al.* (1997) reported a 23% and 15% reduction in body weight and feed consumption respectively in broilers reared under high temperature conditions when compared with their counterparts reared under temperate conditions. Excess dietary Cl^- depresses blood pH, significantly decreases laying performance, and eggshell quality (Gezen, Eren and Deniz, 2005).

Therefore, the dietary manipulations of electrolytes and their salts through the concept of dietary electrolyte balance (dEB) are most beneficial to birds under severe heat conditions (Ahmad and Sarwar, 2005). Formulating poultry diets to an optimum dEB using sodium salts of NaCl or $NaHCO_3$, had resulted in improved body weight gain, feed intake, feed conversion ratio, increased water intake, and reduction in mortality (Mushtaq *et al*., 2013). Borges *et al.* (2003) demonstrated that 240 mEq/kg dEB optimized body weight gain and feed conversion ratio of broiler chicken in hot summer conditions. However, the dEB status of most commercial broiler and layer feeds in Nigeria sampled were widely varied, and below the recommended values which could lead to depress growth performance especially during hot weather periods of the year (Unamba-Oparah *et al.*, 2017). According to Ukwu (2013), broilers placed under low dEB diets during the early starter phase of life consumed 48.9% less amount of feed and gained 18.6% less weight than the recommended values for broilers. At the end of six weeks of feeding such diets, the birds had eaten a 34.5% feed and 20.8% weight gain less than the recommended values. It is therefore imperative to correct the dEB status of such diets by supplementation with Na^+ or K^+.

(b) Yeast as feed additive: Yeast (*Saccharomyces cerevisiae*) is increasingly being utilized as a growth promoting additive in poultry and other animals. Various studies have shown that the supplementation of yeast in diets enhanced growth and feed utilization in broilers (Hana *et al.*, 2015; Ahiwe *et al.*, 2020). Its inclusion in poultry feeds could improve digestibility of vegetable proteins, and fibrous matter in rations. Yeast supplementation in high fibre chicks' diet, improved nutrient retention, while significantly reducing the cost of feeding (Oyedeji, Ajayi and Egere, 2008). The improvement in performance associated with yeast inclusion in poultry diets could be due to improved digestion resulting from improved digestive enzymes activities, manipulation of GIT microflora, and stimulation of immune responses (Kabir, 2009; Zhang *et al.,* 2020)

(c) Vitamins and minerals: Minerals are dietary essentials to all classes of livestock and therefore perform structural, physiological, catalytical and reproductive functions in animals (Suttle, 2010). They are inorganic substances, mostly found in body fluids and tissues, and therefore pivotal in the physiological, biochemical, and enzymatic activities leading to improved growth, egg production and feed – gain ratio (Esonu, 2015). As a premix in poultry feeds, multivitamin – minerals supplements have been used to increase feed utilization, and growth performance, especially in broilers under poor health (Peric, Zikic and Lucik, 2009), and extreme weather conditions (Lin *et al*., 2006). They have been known to enhance appetite, FCR, and immune system, while regulating intestinal microflora in poultry (Dhama, *et al.,* 2014). Supplementation of micro minerals such as zinc has been reported to have beneficial effect in piglets by influencing intestinal microflora (Bednorz, Oelgeschlager, Kinnemann, Hartmann, and Neumann, 2013), while iron plays a dual role as a growth inhibitor and promoter (Visca, Bonchi, Minandri, Frangipani, and Imperi, 2013). Dietary phosphorus plays a key role in body weight gain in broilers (Abudabos, 2012). Therefore, imbalances of these nutrients will result in metabolic disorders, poor growth, low egg production, and hatchability of incubated eggs (NRC, 1994).

Vitamin C as an antioxidant is reported to reduce excessive weight loss in birds due to heat stress (Sahin, Onderci, Sahin, Gursu, and Kucuk, 2003). Its supplementation improves amino acid metabolism and mineral absorption (intestinal iron absorption) required for the synthesis of hormones (Engelking, 2015). Again, a combination of ascorbic acid and L-arginine when supplemented, has been suggested to proffer better meat quality (Dhama *et al.,* 2014). Vitamin E supplemented diets have also been implicated in better growth performance, through improved feed conversion efficiency, immune function and increased vitamin E content in animal meat (McDowell, 1989).

2.6.3 Additives that enhance feed nutrient utilization and uptake

(a) Betaine: Betaine is a trimethyl derivative of the amino acid, glycine which is widely found in nature and synthesized by a variety of plants and organisms (Boch, Nau-Wagner, Kneip, and Bremer, 1997). The origin of the name betaine comes from the plant (sugar beet) from which it was first isolated (Filipčev, Brkljača, Krulj, and Bodroža-Solarov, 2015). There is a growing interest in the use of betaine as feed additive in poultry diets due to its sparing effect on the essential amino acid, methionine, B complex vitamin, and choline (Mahmoudi, Azarfar, and Khosravinia, 2018) by donating its methyl group (CH_3). This, therefore, decreases the requirements of methionine, and choline in the feeds which further brings down the production cost of compounded feed without negatively vitiating the productive performance of poultry (Amerah, 2015).

Methionine, and choline are methyl donors that play significant metabolic roles in animal physiology, and are chiefly involved in the synthesis of proteins, and phospholipids. A reduction in methionine content of poultry diets affects performance, and egg characteristics (egg weight, and egg mass) in laying hen (Cadirci, Koncagul, and Mizrak, 2014). Dietary inclusion of betaine can result in synthesis of three times more methionine than choline and can replace about 25% of methionine without affecting broiler chicken performance (Ratriyanto, Mosenthin, Bauer, and Eklund, 2009).

Betaine has been widely researched for its performance enhancing, antioxidative, immune-modulatory, lipid metabolism (lipogenic), acid/base-balancing, and osmoregulatory effects (Criag, 2004). Its capacity to improve nutrient utilization, protein synthesis, carcass yield, meat quality, and litter quality in poultry has been investigated (Waldroup and Fritts, 2005).

(b) Organic acids: These are added to feed as organic and inorganic acids to lower its pH thereby preventing its deterioration and contamination by microbes and by extension the gut environment of the chicken (Kum, Eren, Onol, and Sandikci, 2010; Dittoe *et al.,* 2018). They are mostly salts of monocarboxylic acids, and short chain fatty acids such as lactic acid, furric, acetic, propionic, and butyric acids (Hajati, 2018). The use of organic acids in poultry will evoke growth performance responses due to their inhibitory effects on of pathogenic and spoilage organisms, and their metabolites in the GIT (Agboola, Omidiwura, Odu, Popoola, and Iyayi, 2015). Organic acids have increasingly been used to replace antibiotic as a growth promoting feed additive and control microbial contamination in poultry (Hassan, Mohamed, Youssef, and Hassan, 2010; Papatisiros *et al.,* 2013). These acids in their undissociated state can diffuse through the cell membrane of pathogenic organisms where it dissociates into anions and protons, thereby lowering the cytoplasmic pH of the pathogen. Consequently, the organism dissipates its energy to restore normal balance, together with lowered enzyme activity, membrane, and DNA damage (Dhama *et al.,* 2014).

(c) Phytogenic additives: Aromatic plants/ photobiotic have been known to have preservative and medicinal properties, hence they have been used over the years to influence the aroma, and flavour of food. Recently, phytogenic plants like herbs, and spices have been investigated as feed additives in poultry diet to enhance performance (Khan *et al*., 2012), due to their digestive, immune system, and anti-microbial functions. The concentrations of chemicals like alkaloids, flavonoids, minerals, and vitamins, aromatic oils, fatty acids, dietary fibres, protein, and carbohydrates present in certain parts of these plants confer therapeutic properties to them. Some of the few examples of such phytogenic reported for their benefits as in-feed additives for improved growth and egg performance effects in poultry are Garlic (*Allum sativum*), Turmeric (*Curcuma longa*), Thyme (*Thymus vulgaris* L.) Aloe vera, onion (*Allium sepa*), Ginger (*Zingiber officinale*, Rosc.), and Noni (*Morinda citrifolia*) etc., (Sunder *et al*., 2014). Studies have suggested that their inclusion in feed modulates the gut microbiome to influence host nutrition, and health, through improved utilization of feed nutrient (Huyghebaert, Ducatelle and Van Immerseel, 2011).

For example, ginger and curcumin (found in turmeric) enhanced nutrient digestion, and absorption due to their positive effects on gastric secretion, and digestive enzymes (Zhao *et al*., 2011; Khan *et al*., 2012). Black cumin seeds (*Nigella sativa* I.) are known for their richness in protein, energy, and fatty acids, and have been reported to have antioxidative (Guler, Ertas, Kizil, Dalkilic, and Ciftci, 2007), digestion, and appetite stimulative (Gilani, Jabeen and Khan, 2004), and hepato-protective properties (Mollazadeh and Hosseinzadeh, 2014). Abou-Elkhair, Selim and Hussein, (2018), specifically reported improved growth performance, and egg characteristics when fennel, red pepper, and black cumin seed were used individually as feed additives in layer diets.

In addition, the active compounds in red pepper (Capsaicin, and Capsanthin) have been found to exhibit chemo-preventive, and chemo-therapeutic effects (Jancso, Kiraly and Jancso, 1997). Red pepper is a rich source of carotenoids, vitamin C, E, and provitamin A, which are well known for their antioxidative functions (Krinsky, 2001). Red pepper has been used to increase appetite, laying performance, and yolk colour characteristics in poultry (Ozer, Zik, Erdost, and Özfiliz, 2006; Lokaewmanee, Yamauchi and Okuda, 2013). Plants containing carvacrol and thymol had strong antioxidant (Lee *et al*., 2003), and antimicrobial activities (Bassole and Juliani, 2012).

(d) Enzymes: In recent years, one of the standpoints for addressing issues of increased cost of poultry feeds in commercial poultry lies with the addition of exogenous enzymes to poultry rations (Alagawany, Elnesr and Farag, 2018). In-feed enzymes are products of fermentation from fungi and bacteria which are added to poultry feeds to break down certain components of the feed, such as xylans, pentosans, and phytates etc., which ordinarily are much difficult to digest by the avian species.

Non-starch polysaccharides (NSPs) exert anti-nutritive effect in animals by increasing the bulk, and transit properties of intestinal content which consequently reduces the digestion, and intestinal absorption of nutrients (Huyghebaert *et al*., 2011). The cell wall of grains utilized in diet formulation contains some levels of soluble NSP that can entrap large amounts of water, during digestion, leading to increased satiety, reduced feed intake, and growth performance in chicken (Yin *et al*., 2004).

Since, poultry birds do not naturally produce enzymes capable of degrading NSPs, and phytates during digestion, it becomes paramount to include exogenous enzymes to such feeds to break the bonds between sugar units of the NSP, thus significantly reducing the gut content viscosity, feed passage rate, manure output, and nutrient excretion in faeces (Maas, 2019). Vohra, Rastogi, and Satyanarayana (2006) reported reduced P excretion up to 50% when supplemental phytase is added to diets thus, reducing environmental pollution. Xylanase enzyme is useful in the utilization of the NSP component of dietary ingredients, while proteases may enhance the utilization of protein fractions of diets (Freitas, Vieira, Angel, Favero, and Maiorka, 2011).

(e) Antibiotics: Antibiotics are antimicrobial substances used against the growth, and multiplication of bacteria organisms. Its discovery and use as therapeutics in human, and animal medicine have been very ancient. They are either applied therapeutically for disease treatment or sub-therapeutic concentrations (at <200 g/t of feed) in feeds to minimize disease outbreaks in poultry, while increasing productive performance via increased feed utilization, feed efficiency and healthy microbiota (Hassan *et al.,* 2010). This is possible mainly through the antibiotic inhibition of GIT infections, GIT modifications and improvements in the microflora balance within the gastrointestinal system (Torok, Allison, Percy, Ophel-Keller, and Hughes, 2011;

Singh *et al.,* 2013), thereby enabling more energy to be used for nutrient utilization and growth (MacDonald and Wang, 2011).

Antibiotic compounds such as Penicillin, Oxyteracycline, Doxycycline, Virginiamycin, Chlortetracycline, Erythromycin, Avilamycin, Furazolidone, Lincomycin, Enrofloxacin and Neomycin etc. help to inhibit intestinal pathogens from colonizing the avian microflora. They also help to reduce the effects of growth – depressing metabolites produced by microorganisms, while improving nutrient absorption due to increased thinning of the intestinal wall (Huyghebaert *et al.*, 2011). The addition of antibiotics to feed also improved weight gain, increased egg production, and egg hatchability (Upadhayay and Vishwa, 2014). Again, animals on subtherapeutic doses of antibiotics were observed to eat less quantity of feed to gain desired level of growth compared to untreated animals, thereby reducing a substantial cost of production (Emami *et al.*, 2012).

However, it must be kept in mind that human and animal health can be affected directly through residual antibiotic concentrations in meat (Ramatla, Ngoma, Adetunji, and Mwanza, 2017), and more so, through the selection of antibiotic resistant strains of bacteria whose virulence may increase to levels of great concern (Mehdi *et al.,* 2018). According to Diarra and Malouin (2014), antibiotics preparations like the penicillin, tetracycline, aminoglycoside, and amphenicol have been detected in animal food products with serious negative impact on human health. For example, tetracyclines had been reported to interfere with teeth development in young children (Kummerer, 2009), while clenbuterol, has shown its potentials to cause poisoning, and other health conditions in humans (Chan, 1999). It is therefore no wonder that there have been consistent calls for the total ban of in-feed antimicrobials in animals intended for human consumption (McNamee, Cunningham and Elliott, 2013). For this reason, the European Union (EU) has since 2006, completely banned the agricultural use of antibiotics for growth promotion in any animal (Marshall and Levy, 2011). The ban was to clamp down on all antibiotics, intended for prophylactic/metaphylactic use in food animal production as well as imported meat raised with antibiotics by the year 2022 (Patel, Wellington, Shah, and Ferreira, 2020). Similarly, the United States, Food and Drug Administration (FDA), on January 1, 2017, banned the use of antibiotics as a growth promoting feed supplement in livestock (CDC, 2020). The ban required veterinary prescription for the therapeutic administration of antimicrobials important in human medicine, and further set a reasonable limit for their use in animal agricultural by the year 2023 as stipulated in the 5-year plan (FDA, 2020; Patel *et al.*, 2020).

The ban has however, galvanized research and implementation of alternative strategies for preventive control of pathogens in farm animals, to maintain the health and performance of poultry (Diarra and Malouin, 2014).

(f) Probiotics: Probiotics is one of the suggested alternatives to antibiotic feed additives, and is believed to increase feed utilization and health benefits in poultry. They are individual or collection of live microorganisms such as *Lactobacilli spp.*, *Streptococcus spp.*, *Bacillus spp.*, *Bifidobacteria spp.*, *Saccharomyces cerevisiae*, and *Enterococcus spp.* etc. that are administered orally in feed or water to evoke favourable responses on host intestinal microbiota (Mookiah, Sieo, Ramasamy, Abdullah, and Ho, 2014; Alade, 2018). The mechanism through which probiotics exerts its influence in animals may not be fully known. However, reports, suggest that they are (1) Antagonistic towards pathogen bacteria proliferation via the secretion of antibacterial substances (bacteriocins, organic acids and hydrogen peroxide) that inhibit microbial development (Hassanein and Soliman, 2010); (2) Cause competitive exclusion of pathogens from adhering to intestinal mucous membranes, and epithelial surfaces thereby suppressing pathogenic colonization of the GI tract (Pan and Yu, 2014), and (3) Compete with pathogens for nutritious substances, while making nutrients non-available to pathogens (Tellez, Pixley, Wolfenden, Layton, and Hargis, 2012). They have also been reported to exhibit immune-modulatory properties, through manipulation of gut microbiota composition (Dhama *et al*., 2014).

Effective control of enteric bacterial infections using probiotics has been reported especially at early post-hatch in order to establish healthy gut microflora, and reduced gastro-intestinal disturbances (Tellez *et al*., 2012; Makled, Abouelezz, Gad-Elkareem, and Sayed, 2019).

Probiotics could also improve digestion, and utilization of nutrients. Its inclusion in feeds improved feed conversion efficiency in broilers, egg production in layers and immune system response in chicken (Salim, Kang, Akter, Kim, and Kim, 2013; Ahiwe *et al.*, 2020). *Bacillus licheniformis* have been found beneficial in increasing the productivity, and quality of broiler meat (Liu *et al*., 2012). They improved the pH, and colour of meat, chemical composition, oxidation stability, and overall quality of poultry meat (Popova, 2017).

(g) Prebiotics: Prebiotics are short chain polysaccharides, and oligosaccharides which selectively stimulate the growth or activities of specific bacteria in the gastrointestinal tract of host animal. Their supplementation in poultry diets is another method of manipulating the avian gut ecosystem towards improved feed efficiency, and performance in poultry. These non-digestible fibres are considered as substrates for the microbiota which on fermentation produces short chain fatty acids (SCFA) in the gut. The acids produced lower the GIT pH, while promoting competitive exclusion of pathogenic microbes from adhesion sites, and increasing absorption of several minerals in the gut (Fallah and Rezaei, 2013) through improved villus cells and length in the ileum (Baurhoo, Phillip and Ruiz-Feria, 2007). When ingested, the prebiotics alter the caecal microbial composition resulting in changes in the microbial diversity which enhance growth performance (Park, Lee and Ricke, 2016).

These products are metabolized by intestinal bacteria to produce short chain fatty acids like propionate, acetate, and butyrate (Jozefiak *et al.*, 2008). Pathogenic bacteria use lectins on their cell surface to bind to mannan oligosaccharides (MOS) from yeast cell walls on the intestinal epithelial cells to initiate attachment and colonization. The mannans also upregulate the acidic pH conditions of the intestine through increasing lactic acid concentration as well as decreasing activities of harmful enteric bacteria such as *Escherichia coli, Salmonella, Clostridium* etc.

Prebiotics such as FOS on the other hand, serve as a fibre and energy source for certain microbial populations and enhance production of organic acids in the gut (Dhama, Mahendran and Tomar, 2007).

(h) Synbiotics: Synbiotics are products primarily composed of probiotics, and prebiotics combinations which jointly exert influence on GIT health, digestibility, and performances in poultry. Their administration in animal feeds favours healthy microbiota which in turn enhances nutrient availability, and absorption, disease prevention, and immunological response (Malik, Prakash, Srivastava, and Gupta, 2019). They are designed to promote synergistic effects that are often more efficient in counteracting the effect of stress-inducing factors and pathogens in poultry production compared with individual probiotic or prebiotic feeding (Mookiah *et al*., 2014). For example, a combination of MOS, and *Saccharomyces cerevisiae* significantly improved gut health in broiler chickens (Padihari, Tiwari, Sahu, Gendley, and Naik, 2014). Similarly, FOS, and *Enterococcus faecum* reduced the intestinal colonization by *Clostridium perfringens* (Malik *et al.*, 2019). *Bacillus subtilis* and inulin increased colonization of the GIT by beneficial microflora along with increased villi-crypts absorptive area (Abdelqader, Al-Fataftah, and Daş, 2013).

(i) Plant ash: Ash is simply the total mineral content of a diet or forage which is the left-over fraction of a diet incinerated above 500 $^{o}{}_{C}$ for two hours (AOAC, 1995). Wood ash has been found to be rich in calcium, thus, have shown some potentials as a mineral additive supplement to livestock production in the tropical regions (Imbeah, 1999; Van Ryssen and Ndlovu, 2007), while improving fibre digestibility and utilization by ruminants when used in the treatment of low-quality straws (Ramirez, Cruz, and Gonzalez, 1992).

Plant ash from different sources have also been reported as good source of absorbable mineral supplement in the diets of pullets (Okoli *et al*., 2014), and growing rabbits (Iwu *et al*., 2013). The supplementation of coconut shell ash (CSA) at 1g/ kg body weight has been reported to support testicular development and increased serum testosterone concentration in male rabbits, while improving weights of female reproductive tract, ovary, oviduct, and uterus respectively (Iwu, 2013; Ebere, 2013). Ash treatment was reported to improve digestibility, and growth in broilers fed on a diet containing high tannin sorghum (Kyarisiima *et al*., 2004). Blake and Hess, (2014) in their experiment with poultry litter ash (PLA), reported that the substitution of Di calcium phosphate with PLA on a weight/weight basis did not compromise body weight gain, feed intake, feed conversion ratio, or bone development in finisher broilers. PLA was found to increase the digestibility of dietary calcium and phosphorus, and therefore can be used as a P and Ca source for broilers. Similarly, Saccomani *et al*. (2016) reported that firewood ash promoted similar performance and bone development as limestone, and therefore concluded that ash could replace limestone as a calcium source in broiler diets at starter phase of development.

Recent studies using ash derived from palm kernel shell have highlighted its potentials as a mineral supplement in broiler production. At 10 kg/ton of feed inclusion level, PKSA enhanced GIT development in chicks (Ohanaka *et al*., 2017; Ohanaka *et al*., 2018a) and improved intestinal uptake of Ca, Na, Mn, Fe and P as reflected by their reduced concentrations in the faeces (Ohanaka *et al.*, 2018b). However, Oso, Idowu and Niameh (2011) on the contrary reported low calcium availability, leg problems and poor growth performance in broilers fed dietary wood ash as calcium source.

(j) Activated charcoal: Incomplete combustion of agricultural residues (ARs) under controlled oxygen environment will usually lead to the production of biochar or activated charcoal (AC). Activated charcoal is formed by the pyrolysis of organic materials with high volatile matter content in the absence of oxygen and possesses high porosity and surface area (Li *et al.,* 2018). It is believed to have enormous absorptive and bacteriostatic properties in the gut, and at such, it is capable of absorbing gases, anti-nutrients, bacteria, and mycotoxins present in livestock feeds, while supplying some essential minerals to the animals (Khoa *et al.,* 2018; Schmidt *et al.,* 2019).

Investigations conducted on broiler chick's diet showed that incorporation of dietary wood charcoal increased feed intake, weight gain, and feed conversion ratio (Kutlu *et al.,* 2001; Durunna *et al*., 2018). It was further observed to slightly reduce abdominal fat in chicks, while increasing ash content of broiler carcass, an indicator of increased mineral intake, and retention that reflects improved digestive processes at early stages of development (Kutlu *et al*., 2001). A significant increase in the crude protein content of the breast muscles of big tom turkeys was also reported when charcoal was added to their diet (Majewski *et al*., 2002). The odour, and domestic fly count in the faeces of chicks on charcoal supplemented diets were lower when compared to those with no charcoal addition (Durunna *et al.,* 2018). Similar results were observed by Asada *et al*. (2002), and Ritz, Tasistro, Kissel, and Fairchild (2011) who reported reduced moisture content of poultry litter and reduced odour. Ingested activated charcoal binds with the uric acid in the manure when voided thus produces ammonium compound instead of the harmful ammonia (Ritz *et al.,* 2011).

2.7 Agricultural Residues as Precursors for Activated Charcoal Production

Agricultural residues are left over rubbles, waste or agro-processing by-products from crops, fruits, vegetables, meat, and dairy whose economic values may be much less than the cost incurred during their collection, transportation, and processing for beneficial use (Obi, Ugwuishiwu, and Nwakaire, 2016). The composition of such waste largely depends on the type and system of agricultural production. Their constituents are usually in the form of solids, liquids or slurries comprising majorly crop waste (stalks, stubbles, fruits, and vegetables etc.), animal waste (manure, waste feed, and animal carcasses), and by-products of food processing (Okoli, 2020). The intensification of agricultural production has naturally increased the quantities of agro-waste and Nigeria as a country also generates her fair share of these waste from crop and livestock production. With an aggregate crop production of 93.3 million tons of major cash crops, and an estimated 285.1 million tons of crop waste, and manure from livestock yearly, the country tends to generate more quantity of stubbles, straws, chaffs, husks, offals, and animal manure than the actual food crops (ECN, 2008). The quantity of these agricultural residues utilized either as feed for livestock, fertilizer for crop production or heat generation is however negligible with a greater percentage dumped at landfills and/or incinerated due to lack of investment in waste management technologies.

The use of agro-residues as feedstock for activated charcoal (AC) production has received several research and industrial attention. Several studies have demonstrated the utilization of agricultural residues, and wastes such as rice husk (Alvarez, Lopez, Amutio, Bilbao and Olazar, 2014) sawdust (Kini, Saidutta, Murty and Kadoli, 2015), tropical wood (Yusufu, Ariahu and Igbabul, 2012), palm kernel shells (Ma *et al.*, 2017), corn cob (Kana, Teguia, Mungfu, and Tchoumboue, 2011), corn stover (Calvelo Pereira *et al.,* 2014), coconut shells (Bernard, Jimoh and Odigure, 2013; Shaheed, Azhari, Ahsan, and Mohtar, 2015), walnut shells (Yang and Qui, 2010), bamboo (Chu *et al.,* 2013), and straw (Cabeza, Waterhouse, Sohi, and

Rooke, 2018) as promising raw materials for AC production. This is made possible by their cell wall structure, which is composed principally of cellulose, hemicellulose, and lignin with little concentrations of extractives/volatiles and ash (Williams, Emerson and Tumuluru, 2017) and gives them their high heating value. Again, their ready availability at little or no cost as well as their capacity to yield high adsorptive AC with low ash content and considerable mechanical strength (Zarifah, 2010) are positive attributes.

Bamboo is one agro-resource traditionally used as structural material in the construction of buildings in Nigeria due to its height, slender, and tough nature. It is chemically composed of substances such as ethanol–toluene, holocellulose, α-cellulose, lignin, resins, gums, and some other compounds (Mahanim, Wan Asma, Rafidah, Puad, and Shaharuddin, 2011). Its high volatility, and fixed carbon content, but low ash content makes it suitable for AC production (Mahanim *et al.*, 2011). Studies have investigated its use as a precursor in the production of activated charcoal (Edward, Cheung, Vinci, and Gordon, 2010). The presence of special microporous structure in bamboo derived AC increases its adsorption capacity even more than wood charcoal (Zhao, Yuan, Jiang, Shi, and Cheng, 2008).

Palm kernel shell (PKS) is a readily available agricultural industry residue that has found limited application in the Nigerian industry (Ohanaka, 2016). According to Okoroigwe, Saffron and Kamdem (2014), PKS is structurally composed mostly of carbohydrates such as lignin, hemicellulose, and cellulose, which gives it its high calorific value, thus it is used in palm oil processing operations as biofuel, while the resultant ash residue is discarded as waste material. Its relative abundance coupled with its content of low inorganic materials, high volatility, and fixed carbon content makes it a good material for AC production (Marsh and Rodriguez-Reinoso, 2006).

2.7.1 Production of Activated Charcoal

Activated charcoal is a product of pyrolysis of carbonaceous organic materials in the absence of oxygen, which has adsorptive properties (Huwig *et al*., 2001; Wang *et al.,* 2017). It is often in granules, pellets or in powder form when ground. It is black in colour, odourless and tasteless, and has a high absorbance for toxins, gases, drugs, and fat-soluble substrates (Osol, 1975). The adsorptive capacity depends on the porosity, surface area, dose of application, type and structure of raw materials and its production processes. (Diamadopoulus, Samaras and Sakellaropoulos, 1992; Schmidt *et al.,* 2019).

Basically, there are two main steps in the production of activated charcoal. The first step is the pyrolysis of the organic precursor in the absence of oxygen under a predetermined temperature, which is then followed by the activation of the carbonized product as the second step. The activation method could either be physical or chemical activation.

(a) Physical method: Physical activation is the thermal activation process. It generally occurs in two stages: carbonization thermal degradation of the organic raw materials and the subsequent activation of the resultant crude char at high temperatures using steam or CO_2 (Baker, 1998). Carbonization converts organic matter to carbon at high temperature ($500^{o}{}_{C}$ to $900^{o}{}_{C}$), while eliminating hydrogen and oxygen to produce a carbon skeleton possessing a latent pore structure (Amir, 2008). The carbonization stage is immediately followed by the activation process, which is carried out by exposing the char product to an oxidizing agent that greatly increases the pore volume through the elimination of carbon burn-off and volatile products (Baker, 1998). The common activating agents used in the physical method include steam, and carbon dioxide (CO_2), at varying activation temperature ranges between 800 $^{o}{}_{C}$ to 1000 $^{o}{}_{C}$ (Yegareh *et al.,* 2006).

(b) Chemical Method: The production of activated charcoal using the chemical activation method involves a single step pyrolysis method which requires the treatment of the biomass precursor with dehydrating chemical agents, usually acids before carbonization. The process begins with the impregnation or soaking of the raw material with an appropriate chemical reagent which is followed by simultaneous carbonization and activation process (Al-Swaidan and Ahmad, 2011).

The common chemical compounds used for the impregnation of precursor materials are dehydrating agents such as sulphuric acid (H_2SO_4), phosphoric acid (H_3PO_4), zinc chloride ($ZnCl_2$), potassium sulphide (K_2S), carbonates of alkali metal, and metal chlorides (Yorgun and Yildiz, 2015; Tadda *et al.,* 2016). The impregnation of precursor materials lowers the temperature requirement during pyrolysis and restricts the formation of tars thereby resulting in a much richer carbon product with more porosity, and yield (Yegareh *et al.,* 2006; Ioannidou and Zabaniotou, 2006). The resultant char is left to cool and thereafter washed with hot distilled water to remove, and possibly recover the impregnation chemical, while enhancing the porosity of the char (Li *et al.,* 2013).

2.7.2 Production of activated charcoal from animal wastes

The use of Animal waste such as poultry manure, cow and pig dung, bones, and abattoir waste, as fertilizer during crop production, feeding of livestock or the generation of domestic fuel have long been practiced in many cultures. Poultry manure or litter for example is composed of poultry droppings, waste feed, and bedding materials obtained from poultry production kept under deep litter or caged system (Shakya and Agarwal, 2017). It is rich in essential nutrients such as crude protein, minerals, and fibre whose amount, quality, and content may vary depending on several factors such as, species, age and condition of birds, diet type, amount of wasted feed, storage, and handling of manure and litter material used (Mariakulandai and

Manickam, 1975 cited in Amanullah, Sekar and Muthukrishnan, 2010). Studies by Henuk and Dingle (2003) had reported poultry manure to contain 23-28% crude protein, 10 – 13 % crude fibre and 23 - 26% total ash contents. Appreciable levels of minerals have also been reported in poultry litter ash (Blake and Hess, 2014), which makes poultry litter ash suitable for plant, and animal mineral source. Pig dung on the other hand contains about 14% crude protein, 13 - 20% crude fibre, and various levels of minerals such as potassium, phosphorus, zinc, copper, cobalt, silver, magnesium, sulphur, carbon nitrogen etc. (Uzoma, 2010).

Intensive livestock production often generates substantial quantity of manure than its primary products of meat, egg, and milk put together (Sillar, 2000). Again, the traditional application of manure as fertilizers on soil could be a potential threat to the environment due to saturation of certain minerals capable of polluting ground water (Lima, Ro, Reddy, Boykin, and Klasson, 2015; Crippen *et al.*, 2016). Therefore, as a waste management option, animal waste is suggested as a potential precursor material to produce activated charcoal for industrial, and farm use (Shakya and Agarwal, 2017). Again, their reported rich content of cellulose, lignin, high carbon content, and volatile matter increases their heating potentials for activated charcoal production (He, Zhang, Riskowski and Funk, 1999).

The production process starts with the carbonization or pyrolysis of these wastes to obtain higher char yield. The thermal degradation of pig dung during carbonization leads to the loss of volatile gases such as alkanes, alkenes, carbon monoxide, alcohols etc., with an increased propagation of carbon in the residue (Iregbu, 2014). Poultry waste char yields lower carbon content, and high ash content than other agro-residue and plant-based activated charcoals at different pyrolysis temperatures (Lima *et al.*, 2015). Reports have demonstrated the capacity of manure-based activated charcoal to significantly adsorb heavy metal ions (Xu, Cao and Zhao, 2013). This is because the high concentration of phosphorus entrapped within the activated carbon matrix as polyphosphate anions during carbonization cum activation process further increases its adsorption properties (Lima *et al.*, 2015). According to Gerlach and Schmidt (2014), the adsorption capacity of poultry litter charcoal has been noted to efficiently absorb 5 times more water and other specific nutrients/organic molecules (amino acids, fatty acids, proteins, minerals, ammonia, urea, and nitrate). This is especially true when it is used as in-feed additive or as litter amendment because of its ability to lock – in moisture, organic and inorganic nitrogen compounds (Gerlach, 2014).

Blending pig dung with some agro residues such as palm kernel shell and bamboo are considered as flame accelerants and have been shown to improve the combustion value of pig dung during activated charcoal or briquette production (Iregbu, 2014). It is therefore expected that such blends will yield activated charcoal of similar quality as other agro- residues reported in literatures.

2.7.3 Factors influencing activated charcoal yield and properties

(a) Carbonization temperature: The carbonization temperature for AC production has the most critical influence on the percentage yield, and morphology of activated charcoal. Generally, increasing the carbonization temperature reduces AC yield, while producing charcoals of greater quality with increased porosity and surface area (Tadda *et al.,* 2016; Wafiq, Reichel, and Hanafy, 2016). The decrease in the char yield at higher temperature could either be due to the decomposition, and volatilization of the biomass leading to shrinking in the carbon structure (Gimba and Turoti, 2008).

High carbonization temperature also leads to increased ash formation due to progressive concentration of minerals (Olafadehen, Jinadu, Salami and Popoola, 2012). Ash is often considered as impurity capable of reducing sorption capacity of AC (Mahanim *et al.*, 2011). However, it has been reported that excess carbonization temperature will reduce the surface area, and total pore volume of AC, due to the collapse of the micropores (Lopez, Centeno, Garcia-Diaz and Alguacil, 2013; Yorgun and Yildiz, 2015). Thus, the excessive burning-off of the organic materials may outweigh the formation of porosity (Hagemann *et al.,* 2018). Lower carbonization temperature will equally yield low quality ACs with less developed carbon structure, and adsorption capacity due to the incomplete volatilization (Lee *et al.,* 2017) and absence of micropores (Deshmukh, Nalawade, Karbhal, Qureshi and Shelke, 2018).

(b) Activating agent: The Activating agent for activated charcoal production can among other factors, affect its properties. Dehydrating agents as activating agents have been reportedly used in producing ACs with higher carbon yield and porosity, while reducing temperature and time requirement during carbonization (Almeida *et al.*, 2014; Kumar and Jena, 2016). Phosphoric acid often promotes the formation of cross-linked structures during pyrolytic decomposition of the precursor material, which aids the development of micro, and mesopores in the activated charcoal so produced (Puziy, Poddubnaya, Sobiesiak and Gawdzik, 2017). Usually, strong bases (KOH and NaOH), increase the temperature requirement for activation, but produce lower yield of AC with highly microporous structures, and high total surface areas (Fernandez-Ruiz, Bedia, Bonal, Rodriguez and Gómez-Sainero, 2018). Again, the use of $ZnCl_2$, will produce ACs that are not suitable for the food, and pharmaceutical industries due to toxicological reasons (Prahas, Kartika, Indraswati and Ismadji, 2008).

Activation of ACs by oxidation (air or oxygen) has been used to modify the properties of ACs intended for soil amendment efforts (Suliman *et al.,* 2017), even though such production method often results in rapid reactions, and difficult to control (Hagemann *et al.*, 2018). AC activation using CO_2 caused the formation of microporous AC compared to steam activation, which enlarges the existing micropores rapidly leading to wider pore-size distribution especially at higher temperature (Bedia, Peñas-Garzón, Gómez-Avilés, Rodriguez and Belver, 2018).

(c) Impregnation ratio: This is the ratio at which the chemical activating agent required for activation is mixed with the feedstock material before carbonization. It has been implicated as one of the factors influencing the quality of AC particularly its porosity (Prahas *et al.,* 2008). The influence of impregnation ratio increases with increasing dose. Higher impregnation ratios have been reported to cause a decrease in activated charcoal yield due to increased gasification of the surface carbon atom, while low impregnation ratio resulted in higher AC yield (Yorgun and Yildiz, 2015; Ko, Phyo and Ni, 2018). Higher ratios were also found to increase the pore volume, and surface area of AC (Yorgun and Yildiz, 2015). However, the treatment of precursors at higher chemical ratios again, reduces the micropore volume, and widens the existing pores of the AC produced, compared to lower impregnation ratios which caused increased volume of micropores (Bedia *et al.*, 2018). Similar reports of increased microporous structures, using H_3PO_4 at low impregnation ratios have been demonstrated, while higher ratios caused wider micropores and mesopores (Lopez *et al.,* 2013).

(d) Activation temperature: The activation temperature plays a vital role on the characteristics or properties of an activated charcoal. Several studies have identified activation temperature as a major factor affecting the surface area and yield of AC products (Foo and Hameed, 2011). Higher activation temperature reduces the yield of AC during production, increasing its ash, and fixed carbon content due to increased volatilization during the carbonization process (Zarifah, 2010; Ahsan *et al.,* 2014). The increased volatilization due to higher activation temperature often results in higher surface area of AC products due to the formation of new pores. (Tham *et al.,* 2010). According to Srinivaskannan and Bakar (2004), the micropore surface area of activated charcoal rapidly increased at activation temperatures of 300–400 °C but decreased at activation temperatures of 400–600 °C, while the number of new pores increased with increased carbon content (Meytij *et al.,* 2011). The increased pore formation therefore increases the rate and capacity of adsorption.

(e) Nature of material: The nature of raw material feedstock intended for activated charcoal production significantly affects its properties. As a result, ACs produced under similar conditions but from different raw materials may exhibit different adsorption qualities (Mdoe, 2014). The production of activated charcoal products with low ash contents, will require the use of precursor materials with low inorganic content, moderately high volatility, and density (Tadda *et al.*, 2016). For example, agro-residues from nuts such as coconut shell, almond shells and fruit stones with high cellulosic contents will yield granulated activated charcoal (GAC) with predominantly micro porous structures, whereas materials with higher lignin concentrations such as palm kernel shell, grape seeds, and cherry stones will yield ACs with macro-porous structure (Zarifah, 2010). Similarly, soft woody residues will produce ACs with large pores.

2.7.4 Physical characteristics

It's been suggested that quality activated carbon must have minimal moisture, and ash content, but rich in carbon and volatile matter (Marsh and Rodriguez-Reinoso, 2006; Tadda *et al.*, 2016). The ash content is composed of minerals such as silica, alumina, iron, magnesium, and calcium whose presence in ACs are considered impurities that reduces their adsorptive properties (Mahanim, *et al.*, 2011). A good, activated charcoal should also have large surface area, and adequate porosity (Yagsi, 2004). The pore distribution and surface area of AC are important characteristics affecting the chemistry and adsorptive capacity of an AC (Ioannidou and Zabaniotou, 2006). These properties are however affected by the nature of material carbonized, impregnation ratio, and carbonization cum activation temperatures. Charcoal and AC have similar production conditions and properties but differ only by the activation of AC. Hence, activated charcoal has more porosity and requires more temperature to be produced compared to ordinary charcoal.

2.8 Uses of Activated Charcoal

2.8.1 Historical uses and mode of action

In ancient history, the Egyptians (1500 BC), the Romans and ancient Hindu, used activated charcoal and biochar as adsorbent for water purifications and medicinal purposes (Cheremisinoff and Ellerbusch, 1978). It has been used as an elixir to reduce the absorption of toxins from the gastrointestinal tract (Luder, 1947). However, Scheele in 1773 discovered that the absorptive properties of charcoal differed according to the precursor material used for its production (Mdoe, 2014). Charcoal was found to decolorize tartaric acid in 1785 (Inglezakis and Poulopoulos, 2006), and later applied in the refinement of sugar in 1794 (Jankowska, Świątkowski, and Choma, 1991).

The application of charcoal as an additive in animal feed gained attention within the 19th, and 20th century. Charcoal blended with spices (Cayenne, pepper etc.), and digestive bitters (gentian) were used as elixirs to cure digestive disorders, and loss of appetite, while improving milk production (Pennsylvania State College, 1905, cited in Schmidt *et al.*, 2019). AC in feed was generally used to prevent digestive problems such as diarrhoea (Mangold, 1936), bacteria toxin adsorption (Luder, 1947), and reduced oocysts excretion in faeces of pets challenged with coccidia infections (Volkmann, 1935).

Industrial use of charcoal in water purifications (Burdock, 1997), and the extraction of silver and gold (Bandosz, 2006) was also popular. Within the period between 1939 and 1945, saturated carbon was employed in the production of gas devices as protective tools against toxic gas attack especially during the wars (CCI, 2006). Activated charcoal products have been employed in the production of air purifiers, deodorizers for refrigerators, colour removal in food, and pharmaceutical products (Ashford, 1994). Recently, AC has been used as an electrode additive for energy storage devices with high energy density and recycling efficiency (lithium-ion batteries for solar energy storage) (Hu *et al.*, 2020).

The AC products are characterized by unique sorption capacity (Baker *et al.,* 1992), which enables them to selectively adsorb or desorb already adsorbed substances (toxins) during digestion (Gerlach and Schmidt, 2012). Sorption capacity is largely dependent on the physico-chemical characteristics of the AC like its extraordinarily large surface area, pore volume, and the chemical nature of the precursor material used for charcoal production (Diamadopoulos *et al.,* 1992). The huge surface area is enhanced through activation process, which opens innumerable bonding sites capable of trapping organic molecules or substances. The surface chemistry of the AC also influences its sorption characteristics (Daud and Houshamnd, 2010). Activated charcoal surfaces can be negatively or positively charged to attract elctrons in solution or suspension, depending on how they are treated. According to Jankowska, *et al.* (1991), the treatment of AC surface with alkaline will increase the capacity of the AC to exchange anions, while acid treatment of the surface increases its ability to exchange cations. Available reports suggest the ability of ACs to function as geo-batteries and geo-conductors that can exchange or mediate electrons in biochemical processes (Yu, Yuan, Tang, Wang, and Zhou, 2015; Sun *et al.,* 2017). It can also act as a catalyst and be used as electrodes in microbial fuel cells (Konsolakis, Kaklidis, Marnellos, Zaharaki and Komnitsas, 2015).

According to Shi *et al.* (2016), the mineral contents, of AC especially iron, and manganese can electrically support microbial growth as an electron storage material. Redox reactions, especially during microbial decomposition of organic matters in the gut, generates surplus surcharged electrons which must be transferred to electron acceptors, because surplus electrons cannot be stored by cells or organisms (Schmidt *et al.,* 2019). Since most feed degrading reactions within the GIT are facilitated by microorganisms that aid the electrons transfer to biofilms (Kracke, Vassilev and Krömer, 2015), electron mediating or shuttling substances are therefore required to buffer or optimize these reactions by electron sharing between biofilms or microorganisms (Schimdt *et al.,* 2019). The AC is a well-known electron mediating compound among several others such as thionine, tannins, quinone, and humic substances (Liu *et al.*, 2012; Kluepfel, Keiluweit, Kleber and Sander, 2014).

Reports have suggested the inclusion of multiple electrons mediating substances in high-calorific diets required for intensive livestock production. This is because of the supply deficit of electron mediating substances in such diets (Sophal, Khang, Preston and Leng, 2013), which are required to enhance redox activities in the GIT, thereby causing increased feed efficiency (Leng, *et al.*, 2013).

2.8.2 Activated charcoal as feed supplement in animal production

Studies on activated charcoal as additive in animal feed for improved health and productivity of production animals are still on-going. Its application as in-feed additive is aimed at improving feed quality, while increasing intake and production characteristics. This may be due to its content of mineral compounds, coupled with its ability to adsorb, and prevent GIT uptake of toxins, anti-nutrients, and other organic molecules (phenols, tannins, alkaloids, salicylates etc.) capable of disrupting normal digestive functions in animals (Jindal and MahiPal, 1999; Poage, Scott, Bisson, and Hartmann, 2000). Phenols, tannins, alkaloids, salicylates etc. are naturally occurring substances present in plants but often elicit harmful effects when ingested. Report of studies have shown that the influence of these substances can be effectively reduced with activated charcoal treatment before feeding (Ben Salem, Saghrouni and Nefzaoui, 2005; Rogosic, Pfister, Provenza and Grbesa, 2006). This in turn increases the utilization of otherwise toxic crops by animals.

In a ruminant study, Poage *et al.* (2000) reported increased consumption of bitterweed (*Hymenoxys odorata*) known to contain toxic levels of sesquiterpene lactones, by lambs when blended with charcoal without any deleterious effects. Van, Mui and Ledin (2006), also reported increased growth, dry matter intake, and digestibility of proteins in goats fed tannin-rich acacia leaf diet blended with 0.50 g and 1.00 g of bamboo charcoal.

AC effects on poultry performance have also been investigated (Kutlu *et al.*, 2001; Samanya and Yamauchi, 2002). AC supplementation at 1% level improved the utilization of chickpea in poultry diets as it was found to improve weight gain in broilers compared to the control (Sivilai, Preston, Leng, Hang and Linh, 2018). Bakr (2008) supplemented broiler diets with citrus wood-derived AC at 2% level, and reported increased feed consumption, body weight gain, and feed conversion ratio at the starter stage, but no effect at the finisher stage even when higher levels of AC were fed. Dietary Supplementation of hardwood charcoal at 0.3% inclusion in poultry diets improved performance, and carcass characteristics (Majewska, Mikulski and Siwik, 2009). Significant AC effects on the size of villi in the gut have been demonstrated using activated charcoal blend with wood vinegar at 1% inclusion in poultry (Ruttanavut, yamauchi, Goto and Erikawa, 2009; Rattanawut, 2014), and piglets (Mekbungwan, Yamauchi, Sakaida and Buwjoom, 2008). Available reports suggest that increase in intestinal villi height signifies improved surface area for nutrient absorption (Onderci *et al.*, 2006). This therefore explains improvements in daily bodyweight gain, feed-gain ratio, meat quality, and shelf quality of meat products as well as immune responses of pigs fed 30 g charcoal-blend with stevia in their diets (Choi, Jung, Lee, Choi, and Lee, 2012). Studies by Chu *et al.* (2013), found 0.3% activated bamboo charcoal inclusion in the diets of young pigs to induce significant improvements in weight gain, pig fat composition, total serum protein, albumin, and cholesterol, and similar growth effects same as antibiotic promoting substances in fattening pigs. However, doubling the supplementation level to 0.6% caused no significant effect on leucocytes, erythrocytes, haemoglobin, haematocrit, and platelets between the experimental groups, but lowered the cortisol level considerably, indicating lower susceptibility to diseases and stress. Sivilai *et al.* (2018) recorded increased weight gain and FCR in native Moo Lath piglets fed 1% charcoal in its basal diet.

Additive effects of AC against mycotoxin and bacterial contamination of feed have also been demonstrated in several animal studies. Earlier studies by Jindal *et al.* (1994) reported improved feed intake and body weight gains in broilers that received supplemental activated charcoal in diets contaminated with aflatoxins when compared to the control. Supplemental AC significantly reduced rate of aflatoxin transfer to milk (Nageswara and Chopra, 2001), and degree of liver destruction (Hatch *et al.*, 1984 cited by Wilson 2014) in goats suffering from serious aflatoxin B1. It's been suggested that the blending of silage with ACs binds mycotoxin and pesticides and enhanced the number of lactic acid bacteria (Calvelo Pereira *et al.*, 2014). Feeding such blends to animals was able to maintain the gut microenvironment (pH level and microbiota) through controlled lactic acid production (Jean, Alexis, Berrain, and Joseph, 2010), while suppressing pathogenic bacteria proliferation (Ayanwale *et al.*, 2006).

Faecal *E. coli* counts in pig manure were significantly lowered with increase in the number of beneficial bacteria (Lactobacillus) in the gut after feeding activated charcoal (Kim *et al.*, 2017). Choi, Shinde, Kwon, Song, and Chae, (2009) had supplemented 0.3% bamboo charcoal powder in pig ration and found it to suppress significantly, the excretion of coliform bacteria, and *Salmonella* in faeces compared to control diet. Increased adsorption of *Salmonella Typhimurium* in broiler carcass fed increasing amounts of activated bamboo charcoal in broiler diets was also reported (Wilson, 2014).

2.8.3 Activated charcoal vs other adsorbents

Feed adsorbents are binders added to diets in order to arrest toxic substances, plant metabolites, heavy metal contaminants and their associated metabolic effects. They are usually clay minerals such as hydrated sodium calcium alumino-silicate, bentonite, zeolite etc., structurally composed of silicon, aluminium, and oxygen molecules with tetrahedral and octahedral layers (Vondruskova, Slamova, Trckova, Zraly and Pavlik, 2010). Other examples include clinoptilolite, illite, kaolinite, and smectite etc. The surface chemistry of the clay mineral determines the degree of adsorption (Vondruskova *et al.*, 2010). For example, clay minerals having bipolar charges exhibit stronger binding property than the unipolar ones.

Clay minerals have been widely used in poultry diets to inhibit mould growth and improve performance of poultry fed mycotoxin-contaminated diets due to their binding properties (Zhou, Gong, Yu, and Li, 2014). Improved intestinal health in chicken due to their binding properties have also been reported (Thacker, 2013). Studies on the use of activated charcoal as a sorbent for decontamination have also been carried out (Huwig *et al.*, 2001; Mezes, Balogh and Tóth, 2010).

Studies comparing the efficacy of activated charcoal to that of clay minerals for the control of pathogen, found AC to give similar results with conventional binders to reduce the proliferation of disease-causing organisms in poultry without altering the gut microbiota (Prasai *et al.,* 2016). Again, ruminants suffering from fodder poisoning, and whose diets were treated with bentonite took twice as long to recover when compared to activated charcoal treatment (Ozmaie, 2011). The ability of activated charcoal to reduce (76%) the transfer of aflatoxin B_1 contamination in goat milk when compared to that of bentonite (65.2%) have been demonstrated (Nageswara and Chopra 2001). These researchers also reported improved live weight in young goats fed aflatoxin B_1 contaminated diets that were supplemented with charcoal or bentonite (Nageswara, Chopra and Radhika, 2004). Denli and Okan (2007) reported similar results from AC supplementation in diets at 0.25 and 0.5 % that resulted in lower levels of aflatoxin B_1 in the liver and blood compared to alumino-silicate. However, AC failed to reduce aflatoxin B_1 metabolite in milk compared to bentonite when supplemented at 0.25% in Holstein cows' diets (Diaz *et al.*, 2004).

2.8.4 Effects of activated charcoal on poultry performance

The effect of both ordinary charcoal and activated charcoal supplementation on the performance of poultry has been well studied. The addition of activated charcoal in the diets of broilers caused increased intake of feed, bodyweight gain, and feed conversion ratio (Kutlu *et al.,* 2001; Ruttanavut *et al*., 2009). Similarly, Samanya and Yamauchi (2001), in their reports suggested that the inclusion of wood charcoal powder, mixed with vinegar in poultry diet improved intestinal villi development in leghorn chicken which caused improved bodyweight gain. Rattanawut (2014) had reported 1.0% bamboo charcoal powder addition to diets of Betong chickens to cause improved bodyweight gain. Kana *et al.* (2011) also demonstrated the ability of agro – residue based-AC products to induce increased body weights in chicken at 0.2, 0.4 and 0.6% dietary inclusion, while depressing broiler growth at 0.8 and 1% inclusion. Another dose-dependent response was reported in finisher broilers supplemented with coconut shell-derived AC product (Jiya, Ayanwale, Ijaiya, Ugochukwu and Tsado, 2013). The authors observed improved FCR at 0.5% AC inclusion beyond which the gains were reversed. Similarly, the supplementation of poultry manure biochar at 2% caused improvements in FCR, but reduced body weight gain at 4% addition in broiler feed (Evans, Boney and Moritz, 2017). However, Dim *et al.* (2018) observed improved body weights, and FCR in broilers fed with 4% and 6% corn stover charcoal supplement than those fed 2% biochar or the control diet. Significant improvements in bodyweight gain, and reduced incidence of foot pad and hook lesions were recently reported by Albiker and Zweifel (2019), when blends of commercial and wood-based charcoals were supplemented at 0.9% and 1% respectively.

Several reports have also demonstrated the ability of AC feeding to significantly improve chicken meat quality (Kim *et al.,* 2011; Yamauchi, Manabe, Matsumoto, and Yamauchi, 2014). According to Kutlu *et al.* (2001), AC inclusion in poultry feeds improved dressed weight, and ash content, and reduced abdominal fat in broiler carcass, while significantly increasing the crude protein content of the breast muscles of big tom turkeys (Majewski *et al*., 2002). Again, coconut shell-derived AC supplementation at 0.5% level in broilers caused increased relative organ weight of broiler carcass and improved the serum biochemical indices (Jiya *et al.*, 2014).

However, the benefits of charcoal feeding were not observed by Odunsi, *et al*. (2007) who reported reduced performance, and least percentage carcass yield when wood charcoal, and vegetable oil were incorporated in broiler diets. This conforms with the submissions of Kutlu *et al.* (2001) who reported slight reductions in feed intake, weight gain and FCR in broilers during finisher stage of development. Oduguwa, Pirgozliev, and Acamovic (2007) also reported no benefit of charcoal to improve feed digestibility, and nitrogen retention, in broilers fed malted sorghum sprouts, and therefore concluded that charcoal was unable to overcome binding effect of sorghum tannins to other feed ingredients.

Supplementing the diet of laying hen with activated charcoal powder improved egg production (Yamauchi *et al.,* 2010), eggshell quality and strength (Rattanawut *et al.,* 2017). Improved egg weight and quality had been observed with supplemental charcoal feeding of laying hen (Ayanwale *et al.,* 2006). Prasai, Walsh, Midmore, and Bhattarai (2017) reported improved FCR and egg weight with AC supplemented layer diets compared to the control. Even with the linear decrease in yolk colour index with increasing AC addition in the diets, the albumen height, haugh unit, and eggshell quality were maintained by AC diets. Such effects were attributed to the beneficial effects of activated charcoal in stimulating intestinal functions, while decreasing gut pH, preventing the growth of pathogens, while enhancing the digestion and utilization of minerals (Ayanwale, *et al.,* 2006). However, supplementing layer diet with oakwood charcoal failed to elicit any improvement in performance and egg quality in layer hen outside the reduction in the number of cracked eggs in the treated birds (Kutlu *et al*., 2001). Similar findings of reduced incidence of cracked, and damaged eggs through AC inclusion in layer diets have also been reported (Ayanwale *et al.,* 2006; Rattanawut *et al.,* 2017).

2.9 Enteric Development of Chicken during the Early Post-hatch Period

The first seven days of life after hatch is the most critical period in the life of a broiler chicken for improved digestive efficiency, growth, and sustenance of life (Willemsen *et al.,* 2008). Post-hatch chicks have limited digestive, and immune system functions required for adequate utilization of exogenous nutrients (primarily carbohydrate and protein-based), and therefore must rapidly undergo substantial physical, and functional GIT and organ development as well as the development of active immunity (Uni, Noy and Sklan, 1999). It has been reported that avian species with high growth rate are characterized by rapid early development of GIT, and digestive organs (Lilja, 1983, cited by Lilburn and Loeffler, 2015). Croom *et al*. (1999) reported that at this early stage, intestinal growth represents most of the body weight increase. Similar increase in proportional growth of the small intestine relative to body weight gain has been reported for both poults and chicks (Sklan, 2001). Studies conducted by Ukwu (2013) showed superior GIT weights development compared to bodyweight gain in broilers fed for 96 hours. At the end of this period, GIT increased in weights by 82.97%, while the body weight increased by 61.27%. This increase in GIT weights according to Yadav *et al*. (2010) is driven more by gizzard, proventriculus, and small intestine weight increases, than crop and large intestine increases. Weights of gizzard, proventriculus, and small intestine, increased rapidly in relation to body weight than other organs and tissues during the early stage of life of the broiler chicken (Noy and Sklan, 1997). This is to ensure development of nutrient supply functions, which are necessary for subsequent growth of demand tissues, such as muscle, skeleton and adipose. Sell, Angel, Piquer, Mallarino and Albatshan (1991), and Uni, Platin and Sklan (1998) had reported a 4-fold increase in the relative weights of the small intestine between hatch and 4 days of life in turkey poults, while duodenum matured much earlier than the jejunum and ileum (Uni *et al.,* 1999).

An increase in gut weight is essential to the secretory activities of pancreatic enzymes to achieve maximal growth in birds during the early growth stage (Lu, 1999; Noy and Sklan, 2001). Nitsan, Ben – Avraham, Zoref and Nir (1991) reported that allometric growth of the pancreas reached a maximum of approximately four times that of the body weight growth at 8 days of age and thereafter declined to approximately 2.5 times at day 23. Similarly, the relative liver weight increased faster than the body weight within the first two weeks post-hatch, (Nir, Nitsan, and Mahagna, 1993).

The length of the small intestine increases with age, with intestinal villus height increasing rapidly immediately after hatch. Crypt depth, which reflects enterocyte maturation rate increases linearly in both duodenum, and jejunum until 10 – 12 days (Uni, Noy and Sklan, 1995). This increases the surface area of the gastrointestinal tract to facilitate increased nutrient uptake by the growing bird (Moran, 1985). An increase in crypt depth, villus height and villus surface area in chicks between post-hatch period and 21 days of age was reported by Iji, Saki and Tivey (2001). They also observed a highly developed gut mucosal structure at hatch, with gross changes occurring in the mucosal structure over the 21 days, which they attributed to exposure to dietary nutrients. Cell proliferation was rapid, and cells also migrated rapidly up to the villus, thus suggesting a rapid response to short period of exposure to dietary factors. However, GIT developments were tied to immediate access to quality feed and water which caused significantly higher relative GIT weight at 30 hours post hatch than those held without food for the first 24 hours (Ukwu, 2013). Feed intake therefore becomes paramount in early digestive organ development and therefore influences the growth, and efficiency of the fast-growing modern broiler (Ukwu, 2013).

The interactions between diet, microbiota, and the gut are pivotal in early intestinal development in chicken (Lan, Verstegen, Tamminga and Williams, 2005). It has been established that gut microbiota influences intestinal development through modifications of villus height and crypt depth (Forder, Howarth, Tivey, and Hughes, 2007), which are suggestive of gut development, health, and functionality (Wang and Peng, 2008). The production of SCFAs as a result of microbial fermentation of dietary fibres aids increased gut weight in chicken through increased enterocyte growth and proliferation (Blottiere, Buecher, Galmiche and Cherbut, 2003). Dietary supplementation of probiotics and prebiotics (fructo-oligosaccharide, and mannan-oligosaccharide) have been found to increase intestinal villus height, and villus-crypt ratio in chicken (Sun *et al.*, 2013) as a result of the manipulation of gut microbiome structure (Pan and Yu, 2014; Reed, Neuman, Glahn, Koren, and Tako, 2017), and mucin composition (Tsirtsiko, Fegeros, Balaskas, Kominakis, and Mountzouris, 2012) as against the direct effect of the feed. Therefore, attention must be paid to the quality of feed offered to chicks at the early stage of development to maximize their growth potentials.

2.9.1 Enteric pH of chicken

Digesta pH is one factor within the gut, that has been shown to influence microbial population, and composition within the gut as well as the digestibility, and absorption of nutrients (Pang and Applegate, 2007; Hajati and Rezaei, 2010). Therefore, maintaining optimal gut pH is critical to improved performance and health of poultry. According to Bristol (2003), abnormal pH ranges at different segments of the gut may have serious consequences on digestion, and mineral absorption. Therefore, an accurate determination of digesta pH in chickens could act as an index for optimum nutrient absorption, and gut health.

The gut system of the chicken is rapidly populated by a diverse community of commensal bacteria shortly after hatch. However, the proximal GIT (crop, proventricular gizzard), and the small intestine is particularly dominated with acid–producing bacteria which are responsible for lowering the pH of crop content (Rynsburger, 2009). Rynsburger (2009) reported a pH of 5.01, and 6.02 for the crop content of broiler chicks at 2, and 15 days of age respectively, while

Ohanaka *et al.* (2018a) reported crop pH range of 5.0 – 5.04 for broilers at 7 days of age. Crop pH ranges between 5.0 – 6.0 have also been reported in adult broilers at 42 and 70 days of age (Paul, Halder, Mondal, and Samanta, 2007; Mabelebele, Alabi, Ng`ambi, Norris, and Ginindza, 2014), signifying increased crop content pH with age.

Again, hydrochloric acid produced in the proventriculus further reduces the pH of the digester. Similarly, the digesta pH further decreases in the gizzard of the bird. This is because of the gastric acid present in the gizzard which mixes with the ingested food to reduce its pH content. It has been reported that the proventriculus and the gizzard in birds are acidic in order to aid in protein digestion by exposing the peptide bonds of ingested proteins for enzyme hydrolysis (Rynsburger, 2009). A low gizzard pH improves activation of pepsin, nitrogen retention, and increased solubility and absorption of the mineral fraction of feed, in the upper part of the gut (Incharoen and Maneechote, 2013; Mabelebele *et al.*, 2014). Rynsburger (2009) in his study reported a proventriculus content pH of 5.20 at 2 days of age, which linearly decreased to 3.37 as the bird aged (15 days). Similar reductions (3.49 – 3.27) were also reported by the author for gizzard content pH values as the birds aged. However, Ohanaka *et al.* (2018a) reported proventriculus (5.60 – 5.70), and gizzard (2.97 – 3.18) content pH ranges for broilers at 7 days of age. Earlier studies by Bowen and Waldroup (1969) recorded pH reductions in the proventricular (3.75), and gizzard (2.47) content digesta of a 19-day old broiler which also conformed with the findings of Paul *et al*. (2007) for proventricular (2.70), and gizzard (3.20) pH of adult broilers at 42 days of age. It seems that the production and secretion of gastric acid by the proventriculus is limited in young chicks while it increases with age (Rynsburger, 2009).

However, in the small intestines, the secretions of bicarbonates increase the pH of the digesta. This is reflected in the works of Rynsburger and Classen (2007), and Paul *et al*. (2007) who reported higher small intestinal pH of 8.15 and 7.5 at 6 and 42 days of age respectively. Similar values (7.02 – 7.29) were also reported by Ohanaka *et al.* (2018a) for 7-day old broilers. Adult broilers usually had lower small intestinal content pH of 6.43 (Mabelebele *et al.,* 2014). Again, at 15 days of age, the digesta content of the different segments of the small intestine of broilers have been shown to record a pH range value of 6.4 - 8.15 (Rynsburger, 2009). Several authors have also shown normal duodenal pH range to be between 5.7– 6.5 (Pang and Applegate, 2007; Walk, Bedford, and McElroy, 2012). The large intestinal content pH has also been shown to have pH ranges between 6.40, and 6.54 for broilers at starter phase and maturity (Mabelebele *et al*., 2014; Ohanaka *et al.,* 2018a).

2.10 Enteric Ecology of Chicken

There are interactions between the chicken gut, its microflora, and the biophysical environment (diet/nutrition, litter etc.). The chicken gut is the primary site for complex biochemical, and physiological processes like digestion, absorption of nutrients, immune system development and regulation (Sommer and Backhed, 2013). The gut and its digesta content constitute a dynamic ecosystem that is richly endowed with intricate microbial communities that must co-exist in a symbiotic relationship to induce tolerance, protection from pathogens, and intestinal homeostasis (Pan and Yu, 2014; Broom and Kogut, 2018), thereby conferring performance and health benefits to the chicken.

The bacterial spps are largely in dominance in the gut particularly the anaerobes such as *Bacteroides, Bifidobacteria, Eubacteria, Streptococci, Lactobacilli* and *Enterobacteria* etc. (Xu and Gordon, 2003; Wei, Morrison, and Yu, 2013). The proximal GIT tract (crop, proventriculus, gizzard) is largely populated by lactic acid bacteria (Bjerrum *et al.,* 2006; Abbas Hilmi, Surakka, Apajalahti, and Saris, 2007), which controls the proliferation of other bacteria species that could otherwise be injurious to the host (Morgan, Walk, Bedford, and Burton, 2014). The duodenal, ileal, and caecal microflora are populated by the *Lactobacilli*, *Clostridiaceae*, *Staphylococcus, Eubacteria, Bacteroides*, *Bifidobacteriae* and *Streptococcus*

(Wahud, 2011; Apajalahti and Vienola, 2016), but the amount and composition differed along the digestive tract. *Lactobacilli* is the predominant organism in the ileum, especially in young birds, while the population of Bifidobacterium dominates in older birds (Amit-Romach *et al.,* 2004). However, *Clostridiaceae* are the chief microflora genus dominating the caecum (Wadud, 2011). These bacteria communities within the gut have significant influence in the absorption of nutrients from food, particularly through energy metabolism from dietary fibres (Kogut, 2016), and will compete with pathogens for gut epithelium colonization. Therefore, a favourable gut microbiota will aid optimal performance of chicken, while unhealthy gut microbial balance may promote enteric infections leading to poor growth and increased mortality (Torok, 2016).

2.10.1 Gut microbiome and nutrition

The quality of feed is key to the development and function of the GIT. It has been established that the composition of diet/nutrition can significantly alter the composition, and relative abundance of intestinal microbiota, while impacting on animal health, and performance (Torok *et al.*, 2011; Borda Monila, Seifert, and Camarinha Silva, 2018). Dietary fibres which ordinarily are undigestible by the host digestive system could serve as substrates, and binding platforms for microbial growth (Deehan *et al.*, 2018), thereby improving energy harvest, and nutrient bioavailability from the diet as well as feed efficiency in broilers (through organic acid production, and non-starch polysaccharide (NSP) digestion (Rinttila and Apajalahti, 2013). Therefore, supplementing additives (enzymes, prebiotics, and probiotics) could help break down NSPs, thus releasing fermentation products on which the microbial members of the gut feed on, to produce short-chain fatty acids (SCFA) required for host nutrition (Borda-Monila *et al.,* 2018). Organic acids are the chief products of microbial breakdown of dietary fibres in the gut (Tazoe *et al.,* 2008), and could serve as metabolic fuel for the proliferation of intestinal epithelium, thereby enhancing the absorptive capacity of the gut for nutrients (Dibner and Richards, 2005).

2.10.2 Gut microbiome and immune system

The interaction between microbial community and host is also known to significantly influence mucosal homeostasis and health status of the host. According to reports, the GI tract is not only a habitat for a diverse community of microbial inhabitants, but also an active immunological organ with more resident immune cells (dendritic cells, macrophages, CD4 T cells, B cells, secretory IgA, IL-17, NK cells, heterophil, etc) than the rest of the body parts (Kogut, 2016). These immune cells are resident in the mucosa, lymphoid tissues, lamina propria and within the epithelium (Brisbin, Gong and Sharif, 2008). Their functions are regulated by the existence of intestinal microbiota whose activities also strengthen the intestinal barrier function (mucus layer, epithelial mono layer, and intestinal immune cells) (Schroeder, 2019).

The mucosal barrier is believed to provide the first response to invading pathogens, and at the same time keeping the resident microbial community in homeostasis (Arrieta and Finlay, 2012; Oakley and Kogut, 2016). Should there be a breach of the barrier, the immune cells are recruited to infection sites to engulf the invading pathogens through oxidative burst (Brisbin *et al.,* 2008).

The epithelial cells of the intestine also contribute to mucosal immunity like the immune cells (Hormann *et al.,* 2014). This is chiefly mediated by the presence of the enteric microbiota required for normal development and production of intestinal mucus on the epithelial mucosa (Jha, Fouhse, Tiwari, Li, and Willing, 2019). Mucins are the main components of the mucus layer, secreted by the goblet cells of the gut epithelium, which in turn supports intestinal microbiota (Shanmugasundaram and Selvaraj, 2012). They are largely aqueous mixture of electrolytes, enzymes, sloughed cells, and glycoproteins (Satchithanandam, Vargofcak-Apker, Calvert, Leeds, and Cassidy, 1990), and an essential energy source for some gut bacteria (Pan and Yu, 2014). The mucus layer covering the intestinal epithelium forms a physical barrier between the mucosa, and the resident microbes (Biasato *et al.,* 2019).

Antimicrobial peptides present on the intestinal epithelial surface are important components of the immune system capable of destroying intestinal pathogens through the disruption of cell membrane permeability, and eventual cell death (Derache *et al.,* 2009). Bacteriocins are examples of antimicrobial peptides produced by bacteria (Dobson, Cotter, Ross, and Hill, 2012), whose activities selectively inhibit the proliferation of other bacteria. A good example is the *Lactobacillus salivarius* which produce bacteriocins inhibitory to *Salmonella enteritidis* and *Campylobacter jejuni* (Svetoch *et al.,* 2011; Messaoudi *et al.,* 2012). Similar effects were shown against oocysts of poultry *Eimeria spp* by bacteriocins from strains of *Enterococcus faecium* (Strompfova, Lauková, Marcinákova, and Vasilková, 2010). Dose-dependent increase in feed conversion ratio, and growth performance in broilers fed dietary nisin (peptide) were reported due to its effect on GIT microbiota and decreasing counts of *Bacteroides* and *Enterobacteriaceae* (Jozefiak *et al.,* 2013).

Probiotics have been reported to deceive pathogenic bacteria to attach to them, and subsequently excreted before they can attach to the mucosa to cause infection (Gibson and Roberfroid, 1995). They also compete with pathogens for adhesion sites at the epithelium (Salminen, Isolauri and Salminen, 1996). The short chain fatty acids (SCFA) produced during polysaccharide breakdown by commensal bacteria are inhibitory to pathogens proliferation due to their lowering effect on the pH within the GIT medium. SCFAs are also required as metabolic fuel for the intestinal epithelium (Stecher *et al.,* 2007), and its production by gut microflora stimulates mucus production and release (Gibson and Roberfroid, 1995).

2.10.3 Effect of AC on gut ecology and faecal characteristics

The effect of AC supplementation on the ecology of the GIT has been investigated. Studies have shown that AC inclusion in poultry feeds could modify the microbiota of the bird through reduction in the pathogen loads in the GI tract without significantly altering the gut microbiota (Toth and Dou, 2016; Prasai *et al.,* 2016). Rattanawut *et al.* (2017) reported decreased population of ileal *E. coli* and *Salmonella spps* in laying hen supplemented with a bamboo charcoal powder- vinegar mix in their diets when compared to the control. Similarly, Watarai and Tana (2005) in their reports, also concluded that the inclusion of activated charcoal blended with wood vinegar in diets of white leghorn significantly reduced intestinal *Salmonella* in the birds, while causing the proliferation of healthy normal microbiota (*Enterococcus faecium* and *Bifidobacterium thermophilum*) in the GI tract.

Willson *et al.* (2019) also demonstrated the efficacy of AC to cause significant reductions in bacteria (*Gallibacterium anastis* and *Campylobacter hepaticus*) colonization of the GIT, particularly at 1 and 2% inclusion beyond which the benefits were not felt or slightly reversed. This is similar to the reports of Prasai *et al.* (2016) who supplemented layer diets with biochar at 4% level and observed reductions in the intestinal proliferation of *Campylobacter jejuni* subsp. and *Helicobacter*. It appeared that lower doses of AC were more effective in reducing pathogen proliferation than at higher dosage. Apparently, the prolonged addition of AC in poultry diets could modify or reduce the composition of beneficial microbiota in the GIT (Naka *et al.,* 2001) as well as essential feed nutrients, and drug absorption (Fujita *et al.,* 2012; Prasai *et al.,* 2017). It also reduced organochlorine (dioxins, dibenzofurans, and biphenyls) concentrations in breast muscles (91%), abdominal fat (99%), and eggs (98%). Similar effects of reduced fluoroquinolone antibiotic (enrofloxacin) concentrations in breast muscle were reported for broilers receiving 2% charcoal in their diets, indicating the efficacy of charcoal feeding in treating antibiotic overdoses (Abd El-Aty, Choi, Park, and Shim, 2007).

The capacity of AC to modify poultry litter, and faecal characteristics have been reported (Asada *et al.,* 2002; Yamauchi *et al.,* 2010; Kammann *et al.,* 2017). Its inclusion in feeds or litter caused reductions in free faecal moisture content, odour, nutrient loss, ammonia, and greenhouse gas emissions (N_2O and CH_4) via volatilization or leaching, be it in fresh form or during composting (Borchard *et al.,* 2019; Sha, Li, Lv, Misselbrook, and Liu, 2019; Rong, Zheng, Zhang, Yang, and Li, 2019). Similar findings by O'Toole *et al.* (2016) suggest that blending straw or sawdust beddings with AC at 5 – 10% volume decreased the incidence of hoof disease, odour, and nutrient losses.

2.11 Development of the Egg

2.11.1 Anatomy and physiology of the avian female reproductive system

The avian female reproductive system primarily consists of the ovary, and oviduct (Nys, 2011). These reproductive organs largely occupy a greater portion of the chicken's abdominal cavity. In poultry, only the left ovary, and oviduct are functional. This is because, the right ovary and oviduct regress during post-embryonic development, leaving only the left ones to mature (Johnson, 2015).

(a) Ovary: The ovary (Figure I) is a bunch of developing follicles which are grossly flattened in young immature birds but gradually increases in size to about 150 g and accumulates yolk materials as the bird attains reproductive age (Nys, 2011). A fully developed ovary has a grape-like clusters of developing yolks sequentially lined around the ovary in the order of development or hierarchy. It has two distinctive parts namely, the medulla, and cortex firmly attached to the body wall by a thin ligamentous structure called the mesovarium (Pollock and Orosz, 2002; Johnson, 2015). The primary functions of the ovary are to produce ova as well as the reproductive hormones such as oestrogen, progesterone, and sometimes testosterone. The surge in the secretion of gonadotropic hormones of the pituitary causes the rupturing of a mature follicle towards releasing mature ova into the oviduct during the ovulation process (Nys, 2011).

(b) Oviduct: The oviduct (Fig. I) is a thick twisted and muscular tubular structure of about 68-70 cm long in the sexually mature hen, but is less prominent, thin, and narrowly structured in the immature or non-breeding hen (Nys, 2011; Rahman, 2013). Shelled eggs are solely formed in the oviduct. In the sexually active bird, glandular development results in the thickening of the oviductal walls which eventually differentiate into five functional segments associated with egg formation. The segments include the infundibulum, magnum, isthmus, uterus, and vagina.

(i) Infundibulum: The infundibulum (Fig. I) is the first section of the oviduct with an entire length of about 10 - 12 cm long (Rahman, 2013; Aro, 2019). The funnel opening is an elongated slit directly facing the ovary which is positioned to engulf an ovulated secondary oocyte liberated from the largest follicle. Fertilization of the ova occurs in the funnel-shaped portion of the infundibulum (Johnson, 2015; Rahman, 2013). Sperm have been observed to be stored in the tubular glands of the infundibulum (Bakst, Wishart, and Brillard, 1994; Orosz, 2012). The tubular region is also described as the chalaziferous zone where the formation of chalazae is first initiated. The region also secretes a thin chalaziferous layer that immediately surrounds the yolk (Rahman, Baoyindeligeer Iwasawa, and Yoshizaki, 2007). In the chicken, the ovum passes through the infundibulum within the range of 15 - 30 minutes (Johnson, 2015; Mishra, Sah and Wasti, 2019).

(ii) Magnum: The magnum (Fig. I) is the second segment of the oviduct following the infundibulum where thick albumen protein is secreted round the ovum (Pollock and Orosz, 2002). It is the longest, and most coiling part of the oviduct with an estimated length of 33-37 cm (Nys, 2011; Rahman, 2013; Aro, 2019), and morphologically differentiated into tubular gland cells, ciliated cells, and goblet cells. The magnum is also heavily populated with large number of secretory cells in the epithelium, and tubular glands compared to the other segments. The tubular glands control the production of ovalbumin, lysozyme, and conalbumin under the regulation of oestrogen, while the goblet cells synthesize avidin following exposure to progesterone, and oestrogen (Tuohimaa *et al.,* 1989). The magnum differs from the infundibulum by its possession of thick muscular walls, and larger external diameter, caused by the presence of numerous glands within its massive mucosal folds (Rahman, 2013). The secretion of albumen protein by the branched tubular glands is responsible for the increased thickness of the mucosal folds. The yolk membranes are strong and permit considerable squeezing as the ovum is further moved because of smooth muscle contractions. The ovum remains in the magnum for approximately 2-3 hours in the chicken (Johnson, 2015).

(iii) Isthmus: The isthmus segment (Fig. I) is short, and about 10 cm long with less prominent mucosal folds (slightly constricted) compared to the magnum (Nys, 2011; Aro, 2019). It is subdivided in two parts viz: the upper white, and the lower red isthmus where the inner and outer shell membranes are formed, as well as an additional deposition of protein on the albumen (Ottinger and Bakst, 1995). The distal portion of the isthmus is also responsible for initiating calcification of the eggshell (Rahman, 2013; Johnson, 2015). The movement of egg through this short segment of the oviduct takes approximately 1-2 hours in chickens (Pollock and Orosz, 2002).

(iv) Uterus: The uterus or shell gland (Fig. I) is the segment immediately succeeding the isthmus. It is about 10 -12 cm long (Nys, 2011; Aro, 2019), and has the same thickness with the isthmus except for an enlarged pouch at the distal end, where egg is withheld for hours (about 20 hours) during eggshell formation (Nys, 2011). The eggshell is largely made of calcium carbonate which must be recruited from the hen’s bone for the eggshell formation. Bone calcium provides nearly half of the calcium required for eggshell formation, while the rest of the calcium needs is supplied from the hen’s diet. Egg shell pigmentation also occurs in this region during the remaining period (about 3 minutes to 3 hours) before oviposition (Johnson, 2015).

(v) Vagina: The vagina (Fig. I) is the short (10-12 cm long), and last portion of the oviduct proximal to the cloaca. The vaginal mucosa is bereft of secretory glands, and thus not directly involved in egg formation. However, its possession of powerful muscles within the vaginal walls and especially at the utero-vaginal junction work simultaneously to expel the newly formed egg out of the hen’s body (Pollock and Orosz, 2002; Rahman, 2013). Again, the linings of the utero-vaginal junction act as ‘sperm nests’ for the collection and prolonged storage of sperm (Baskst *et al.*, 1994).

The vagina also facilitates the deposition of egg cuticle on the egg prior to oviposition**.** The cuticle is a thin waxy shield composed of polysaccharide, lipid, and protein unevenly distributed over the entire egg surface, with the primary function of protecting the egg against water vaporization, and microbial invasion (Johnson, 2015). The cuticle is formed during the last 30 minutes prior to oviposition.

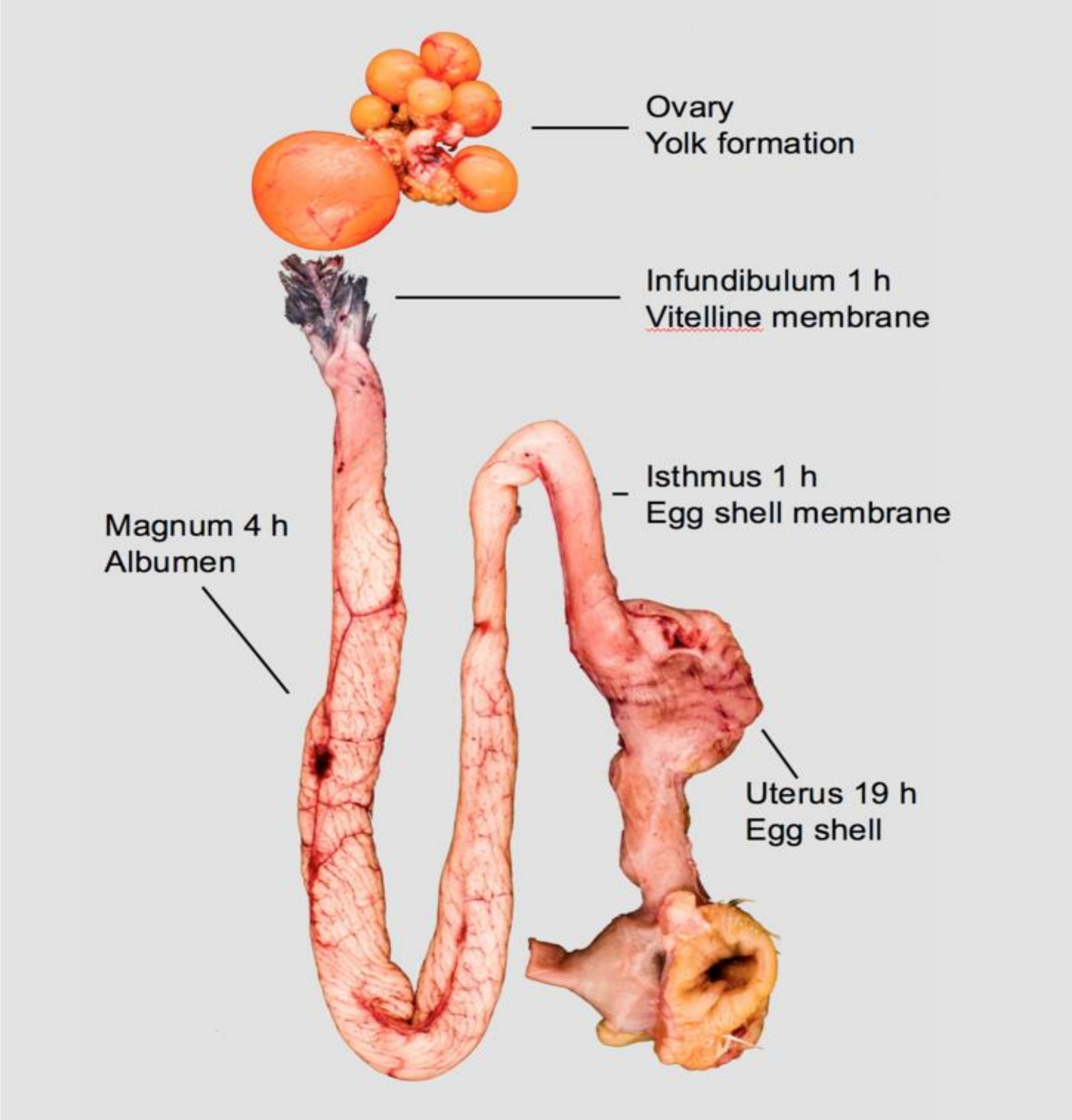

Figure I: Process of egg development in the oviduct (Source: Kaspers, 2016)

2.11.2 Egg development and oviposition

During the embryonic development of the domestic hen, the ovarian follicles contain several thousands of oocytes which subsequently undergo reduction division to form first order oocytes shortly after hatch (Nys, 2011). The first-order oocyte undergoes extensive morphological, and chemical changes/development such as increase in size and accumulation of yolk to become a secondary oocyte (Pollock and Orosz, 2002; Nys, 2011). These changes begin as early as the differentiation of the oocyte during incubation and continue up to adult age of the hen shortly before ovulation. However, only a limited number of these oocytes will subsequently develop to form yolk or pre-ovulatory follicle through the accumulation of lipids (triglycerides, phospholipids, and cholesterol), and protein compounds, chiefly synthesized by the liver (Johnson and Woods, 2007; Nys, 2011).

(a) Egg development: The development, and maturation of ovarian follicles, however, follow a hierarchical arrangement or sequence which guarantees the almost daily production of an egg (Ottinger and Bakst, 1995). This is because of the serial development of oocytes selected to ovulate successively at daily intervals as influenced by the hypothalamic-pituitary regulated hormones (Bentley *et al.,* 2007; Johnson and Woods, 2007).

Gonadotropins such as luteinizing (LH), and follicle stimulating hormone (FSH) are considered the major hormones responsible for ovarian, and follicular development and stimulates the secretion of steroid hormones such as testosterone, oestrogen, and progesterone (Ottinger and Bakst, 1995). Progesterone secreted by the large follicles stimulates the preovulatory surge in gonadotropins. FSH mediates the initial recruitment, and growth of the follicles, while luteinizing hormone exerts its influence on the pre-ovulatory follicle.

Having reached the limit of its growth, the mature (F1) follicle detaches from the ovarian stalk, expelling yolk into the oviduct by the process called ovulation. Ovulation is controlled by the activities of the pituitary hormones. The increased secretion of progesterone by the mature follicle (F1) a day prior to ovulation influences a surge in the secretion of LH by the posterior pituitary gland through a positive feedback mechanism which in turn elicits ovulation (Bentley *et al.,* 2007).

The expelled yolk is engulfed by the infundibulum through its powerful muscle contractions during ovulation. The secretion of albumen, vitelline membrane, and chalazae is initiated in the infundibulum and could take about an hour to form (Pollock and Orosz, 2002; Rahman, Iwasawa, and Yoshizaki, 2007). The chalazae are the thick layered coverings of the egg yolk with spiral filamentous fibres at opposite poles of the egg yolk which maintains the yolk in a central position within the albumen (Nys, 2011). These fibres wind, and twist during the rotational movement of the egg in the magnum which is the main site of albumen protein synthesis as the egg navigates its way through the oviduct.

The albumen is the protein-rich portion of a shell egg consisting of about 60% of the total egg and provides nutritional support during embryonic development. According to Johnson (2015), the major albumen proteins include the ovalbumen (54%), ovotransferrin (13%), ovomucoid (11%), ovoglobulins (8%), lysozyme, (3.5%), α and β ovomucin (1.5–3.0%), and minor concentrations of avidin, flavoprotein- and thiamine-binding proteins, ovomacroglobulin, and cystatin. These proteins are said to possess antimicrobial properties that help to prevent the invasion of micro-organisms into the yolk or the growing embryo (Rehault-Godbert *et al.,* 2013). The production of albumen proteins in the tubular glands of the magnum occurs according to a definite sequence influenced by steroid hormones (Nys, 2011; O'Malley, 1984). According to Muramatsu *et al.* (1991), the amount of protein contained in the walls of the magnum corresponds to that of two eggs, and therefore ensures increased formation of egg albumen. The deposition of albumen proteins is linear, and takes about 3.25 – 3.5 h (Nys, 2011; King and McLelland, 1984). The albumen is initially hydrated (plumping) in the distal part of the magnum, where water, and electrolytes are added to the albumen (Nys, 2011).

The isthmus is the secretory site for the outer and inner shell membranes. In addition, a small amount of albumen protein is deposited to the egg, while providing structural foundation for the initiation of eggshell calcification (Rahman, 2013; Sah and Mishra, 2018).

Then the egg leaves the isthmus for the uterus, where it stays for nearly 20 hours for more hydration (plumping) and shell mineralization leading to the formation of eggshell (Pollock and Orosz, 2002; Nys, 2011). According to Nys (2011), large amounts of calcium, and a small amounts of shell protein such as ovocleidin 17, ovocalyxin 116, and ovocalyxin 32 are swiftly deposited on the egg 10 to 22 h after ovulation but stops about 2 h prior to egg expulsion. The amount of calcium required for eggshell formation is enormous, and must be supplied from the hen’s diet, and in part from the medullary bones (Nys, 2011). Therefore, the supply of large dietary particles of calcium sources, can provide calcium for a longer duration in the gut, thereby reducing the incidence of bone remineralization for shell formation.

(b) Oviposition: On leaving the uterus, the egg enters the vagina which is the conduit from which the formed egg is expelled through the cloaca, and the vent. The expulsion process is termed oviposition. It can be defined as the expulsion of the newly formed shell egg from the oviduct through well-coordinated physiological activities of the abdominal, vaginal, uterine, and utero-vaginal sphincter muscles. At the inception of oviposition, the uterine muscles contract, and propel the shell egg into the vagina through the utero-vaginal sphincter and is largely regulated by hormones such as arginine vasotocin, oxytocin, and prostaglandins (Johnson and Whittow, 2000). The administration by injection of arginine vasotocin elicited uterine contractility, and premature expulsion of the egg (Saito, Shimada, and Koike, 1993). Similar observations were made following the exogenous administration of PGs (PGF 2α) (Takahashi, Tajima, Nakagawa-Mizuyachi, Nakayama, and Kawashima, 2011).

The duration between ovulation, and oviposition is about 24–26 h, while the interval between oviposition, and the subsequent ovulation occurs 15 to 75 min after egg is laid (Sturkie, Hazel, and Wood, 2000; Pollock and Orosz, 2002).

2.12 Characteristics of Quality Eggs

The quality of egg and its content are those characteristics that relate to its size, shell quality, freshness, weight, albumen, and yolk, and their chemical compositions which together affects consumer acceptability, and preferences (Narushin, 1997). Egg quality components can be categorized into external and internal characteristics.

2.12.1 External qualities of the egg: These are those phenotypic or observable features of the shelled egg which can influence or undermine consumer acceptance, and or the integrity of the egg during storage. Quality characteristics such as egg size, shell hygiene, shell strength, shell texture, colour, and egg shape are the primary focus.

(a) Egg size: Egg size or its weight is the primary criterion used in the grading of eggs either destined for table consumption or hatching (Farooq *et al.*, 2001), and are classified in various sizes such as jumbo (≥70 g), extra-large (65 – 70 g), large (56 – 65 g), and medium (49 – 56 g) (FAO, 2003). The variations in the weight of eggs are largely influenced by factors such as the breed of the chicken, the age of hen, and environmental temperature condition of the hen - house. The weight of an egg increases as the hen gets older, (King'ori, 2012; Robert, Chousalkar, and Samiullah, 2013). There also exists a strong correlation between egg weight and hatchability of eggs. According to reports, the availability of nutrient to the neonate chick and the weight of chick at hatch are largely influenced by the size or weight of the egg (Saatci, Kırmızıbayrak, Aksoy, and Tilki, 2005). Again, the percentage of dead embryos is usually higher in over-sized eggs (Zglobica and Wezyk, 1995).

(b) Eggshell quality: Eggshell quality is another important quality trait that also affects commercial egg producers, and hatchability of eggs if not properly managed. The damage to eggs due to poor eggshell quality has financial consequences to the producers. According to Chukwuka *et al.* (2011), external egg quality characteristics are evaluated based on cleanliness, integrity, texture, shape, and colour.

Dirt on eggshell is a hygiene indicator that influences consumer acceptability of shell eggs. Eggs can become soiled due to faecal contamination, contact with dirty equipment or broken eggs, dust, feathers, and stains capable of supporting the growth of potentially harmful bacteria present on the shell, thus compromising consumer safety (Rossi and De Reu, 2011). Therefore, clean eggshells will ensure consumer satisfaction, and dietary safety.

(c) Integrity of eggshell: The integrity of an eggshell has a lot to do with its mechanical strength, and thickness. Measuring the eggshell thickness is a frequently used method for determination of the eggshell strength. The eggshell strength, and thickness are strongly linked to egg size or weight (Hunton, 1995). Reduction in eggshell strength, and the frequency of cracking have been observed to increase with an increase in the egg size in aged hens (Roberts, 2005). This is because of exhaustion effect coming with increased laying and secretion of blood calcium, and phosphorus. Therefore, smaller eggs may possess stronger shells than larger ones (Butcher and Miles, 2003).

(d) Egg shape index: Egg shape index is the ratio of width of a shell egg to its length (Narushin, and Romanov, 2002), and is one of the quality standard central to egg quality determination of table eggs. According to reports, the shapes of table eggs ranged from round, oval, conical, to spherical (Idahor, Akinola, and Chia, 2015). The oval shape of poultry shell eggs is formed as a result of the muscular activities within the oviduct during the process of egg formation, and/or oviposition (King'ori, 2012; Idahor, 2017). Mishappened shell eggs are mostly downgraded since normal eggs destined for the market are generally oval-shaped with a shape index range of 70 – 76 (Zeidler, 2002). A shape index of 74 has been reported as the standard, with higher values indicating a rounder shape, while lower values depict eggs of elongated shape (Romanoff and Romanoff, 1949). Higher shape index positively correlated with increased shell thickness (Altuntas, and Sekeroglu, 2008), and shell strength (Blanco, Icken, Ould-Ali, Cavero, and Schmutz, 2014). Again, abnormally shaped eggs strongly correlate with decreased hatchability of fertile eggs (Narushin, and Romanov, 2002).

Soft shelled or shell - less egg is also another phenomenon which occasionally occurs in young birds due to early oviposition of an egg which is still forming within the uterus (Travel, Nys, and Bain, 2011). The shell membrane is deposited on the yolk and egg white, but the egg somehow side-steps the shell-forming process, leading to the partial or non-deposition of shell on the egg. This condition is not necessarily an indication of health challenge but could be triggered by the deficiencies of important minerals such as calcium, phosphorus, and/or vitamin D required for shell formation. It is also commonly found in laying hen kept in cages compared to those on litter floor (Zeidler, 2002). Increased bird activities or noise especially during egg formation increases the incidence of laying such eggs. However, diseases such as infectious bronchitis, and egg drop syndrome have been shown to increase the incidence of shell - less eggs (Butcher, and Miles, 2003).

(e) Shell texture: Eggs with smooth shells have high consumer appeal and are often favoured over those with rough ridges or textures. Occasionally, the eggshell becomes damaged during shell formation in the shell gland due to pressure mounted on the sides of the bird and the egg itself because of overcrowding (Zeidler, 2003). The damaged egg later gets repaired, and covered with more shell material before oviposition, thus, resulting to ridges on the eggshell (Abanikannda *et al.,* 2007). This phenomenon occurs especially when eggs of larger diameters are being formed and due to the increased adhesion of calcium carbonate particles on the shell surface. Reports suggests that the collection of debris on the shell membrane surface, inside the oviduct will cause rough egg surface formations (Zeidler, 2002). Severe pimpling can cause an egg to be downgraded during candling.

(f) Egg shell colour: The colour of the eggshell will influence consumer preferences thus, an important economic quality parameter (Wei and Bitgood, 1989). Egg shell colour is determined primarily by the genetics of the hens (Jacob, Wilson, Miles, and Mather, 2003), while the intensity of shell coloration is dependent on the presence of pigments, such as protoporphyrin, and or biliverdin in the eggshell (Zhao *et al.,* 2006; Wang *et al.,* 2007). The shell colour has been found to also correlate significantly with shell thickness (Ingram, Hatten, and Homan, 2008), and decreases as the hen ages, due to increase in egg size without a corresponding increase in pigment deposition (Odabasi, Miles, Balaban and Portier, 2007).

2.12.2 Internal quality of the egg: Egg internal qualities are those characteristics relating to albumen, and yolk characteristics.

(a) Albumen quality: The quality of the albumen has a major influence on the interior egg quality of fresh eggs, and it is measured through the evaluation of albumen height, haugh unit (HU) and pH (Rossi and De Reu, 2011). The height of the thick albumen is pivotal in determining the grade of an egg, and together with egg weight, determines haugh unit. Haugh unit is a quality parameter used for the determination of egg freshness. Haugh unit values range from 0 – 130 of which higher numbers signifies freshness, and better quality of eggs (Shi, Wang, Dou, and Yang, 2009). According to USDA (2000), grade AA eggs have HU values of 72 and above, grade A: 71 – 60 HU while B grade has a range of 59 - 31 HU. A typical specification for Haugh units from shell egg is said to be 92.2 (Hy-Line 2016). Therefore, the thinning or poor viscosity of the albumen as a sign of quality loss resulting from egg ageing, elevated temperature, and reduced relative humidity (Li-Chan, and Kim, 2008) grossly affects HU values. Aging of the egg mostly due to prolonged storage causes the ovomucin layer of the thick albumen to grow weaker, thus abnormally increasing the albumen dimensions (albumen length and width) and pH with a consequent reduction of the albumen height (Alade, Usman, and Muhammed, 2013; Khatun, Rashid, Faruque, Islam, and Ali, 2016).

(b) Size of the air cell: The size of the air cell in between the shell membranes at the large end of the egg is another parameter used especially in the US egg industry in the grading of eggs. Air cell size increases in depth, and diameter with increasing age of the eggs under certain conditions such as increased temperature, humidity, and loss of gases. The USDA standards for grading eggs requires that “Grade AA” eggs to have air cell sizes not larger than one-eight of an inch or 0.32 cm in depth; “Grade A” eggs may have air cells of 0.48 cm in depth, while “B grade” could have air cells above 0.48 cm in depth (Zeidler, 2002).

(c) Yolk quality: There are two components to yolk quality, viz: the colour of the yolk, and the strength of the perivitelline membrane. The freshly laid egg has round, and firm yolk which is grossly flattened with ageing of the egg. This is caused by the deterioration of the perivitelline membrane during storage or ageing of the hen. Weak perivitelline membrane will cause the yolk to break more easily, allowing the flow of albumen water into the yolk (Kirunda and McKee, 2000; Jones, Lawrence, Yoon, and Heitschmidt, 2010) whereas yolk colour preferences vary considerably depending on consumer expectations in different countries (Rossi, and De Reu, 2011). These subjective colour demands ranges between a light yellow to a deep orange colour commonly evaluated visually using the La Roche yolk colour scale (1-14) (DSM colour fan). Yolk colour is greatly influenced by various dietary feed components (carotenoids) present in the feed such as additives (Jacob *et al.*, 2003; Hernandes, Beardswort, and Weber, 2005). Hy-Line (2016) had suggested a yolk colour score of 4.0 as the minimum acceptable value.

(i) Percentage yolk: The proportion of yolk in a shell egg is also a quality factor for table eggs. Occasionally, a hen produces double-yolk eggs through the release of two yolks from the ovary in quick succession. This phenomenon is associated with multiple ovulations in young birds coming into lay because of excess stimulation of light, even though genetic factors could also be involved (Zeidler, 2002; Travel *et al.*, 2011). Double - yolk eggs are larger than eggs with a single yolk but are usually not fertile for hatching due to inadequate nutrients content and decreased available space for the development of chicks. Yolk-less eggs can also occur in young birds when the ovary or oviduct shed off a bit of tissue during egg formation.

(d) Blood and meat spots: The presence of blood and meat spots in table eggs are negative quality characteristics (De Ketelaere, Bamelis, Kemps, Decuypere, and De Baerdemaeker, 2004). Blood spots usually form around the yolk with some occasional spill over into the albumen, while meat spots are common with the egg white. Stress factors including toxins and/ or vitamin K antagonists could cause the particles consisting of either blood or lymphatic tissue from a haemorrhaging follicular capillary in the ovary or the oviduct during ovulation to leak into the egg (Zeidler, 2002; Chukwuka *et al.,* 2011). Therefore, the reduction in frequency of these spots is one goal of quality assurance. On the other hand, the presence of blood spots in eggs destined for hatchery is indicative of the growth of an embryo inside, and therefore desirable.

2.12.3 Effect of AC and other absorbents on egg laying and quality characteristics

Feed adsorbents are increasingly important additives that function as binders when added to animal feed in order to arrest toxic substances, plant metabolites, heavy metal contaminants, and their associated metabolic effects. They are usually clay minerals such as hydrated sodium calcium alumino silicate, bentonite, zeolite etc., and charcoal with inherent properties to adsorp or desorp toxins, while improving intestinal health in chicken (Thacker, 2013; Zhou *et al.,* 2014).

Improved egg weight and eggshell quality had been observed with supplemental charcoal feeding of laying hen (Ayanwale *et al.,* 2006). Charcoal addition at 1 – 2% in the feed significantly improved growth, egg production and modified GIT microbial community in the hen (Gerlach and Schmidt, 2012). In another study, the inclusion (2 and 4%) of AC in the diets of laying hen, improved egg weight, FCR, and intestinal villus height, same as hens' fed zeolite and bentonite (Prasai *et al.,* 2017). Such effects were attributed to the beneficial effects of activated charcoal in stimulating intestinal functions, while decreasing gut pH, preventing the proliferation of pathogens, and enhancing the digestion and utilization of minerals (Ayanwale, *et al.,* 2006; Prasai *et al.,* 2016).

Similar findings of improved performance, and egg weight was reported for zeolite at 0.5% dietary inclusion in laying hens (Radović, Rajić, and Radovanović, 2000). According to the authors, adding 0.5% of a commercial zeolite (Min-a-Zel) in T - 2 mycotoxin contaminated feed for laying hens improved egg laying, and mitigated the negative effects of T – 2 mycotoxicosis. Moghaddam, Jahanian, Najafabadi, and Madaeni (2008) increased the inclusion level of zeolite to 1.5% in Hy-Line breed of chicken and reported increased egg production at 36 – 42 weeks stage of production. At 1.5 and 3% inclusion, zeolite again significantly increased shell egg production, egg weight, eggshell thickness, and the eggshell percentage in Hy-Line W – 36 hens (Kermanshahi, Haji, Hashemipour, and Pilevar, 2011). Other absorbents such as clinoptilolite, and bentonite in layer diets increased productivity of laying birds. For example, the addition of 2% clinoptilolite in layer feed significantly increased egg weight in hybrid layers of Bovans Goldline but caused reductions in egg weight at 4% inclusion (Machacek *et al.,* 2010). Hashempour, Kermenshashi, and Pilevar (2010) reported increased egg production compared with the control in laying hen fed diets containing sodium bentonite for over 12 weeks, while Gilani, Kermanshahi, Golian, and Tasmasbi (2013) reported improved hen-day production percentages and egg mass with bentonite inclusion at 1%, compared with the control.

The benefits of commercial absorbents lie in their ability to reduce feed passage rate through the GIT, and the reduction in the population of pathogens, and toxins in the gut, thus, resulting in improved absorption, and utilization of feed nutrient (Placinta, D'mello, and MacDonald, 1999; Tauqir, and Nawaz 2001).

2.13 Palm wine as Probiotics and Organic Acids Source in Animal Nutrition

Palm wine is a fermented alcoholic product from fermented saps of various tropical plants particularly the Palmae family (Okafor, 1978 as cited by Santiago-Urbina, and Ruiz-Teran, 2014). Plants such as raffia palm (*Raphia hookeri*), oil palm (*Elaeis guineensis*), Ron palm (*Borassus spp*), coconut palm (*Cocus nucifera*), *Nypa fruticans* palm, and date palm (*Phoenix dactylifera*) etc., are the commonest tropical trees whose sap are tapped for consumption (Dayo-Owoyemi *et al.*, 2008). In Nigeria, Raphia palm, and oil palm are the two major sources of palm sap (Ezeronye, and Legras, 2009).

The palm wine from these sources has a rich content of sugars, proteins, alcohol, organic acids, minerals, and vitamins (Ezeagu, Fafunso, and Ejezie, 2003; Ogbonna *et al*., 2013; Karamoko, Deni, Moroh, Bouatenin, and Dje, 2016). They are also rich in microbial diversity including yeast (*Saccharomyces cerevisiae, Saccharomyces chevalieri* etc.), lactic acid forming bacteria (LAB) (*Lactobacillus plantarum, Leuconostoc mesenteroides, Lactococci* etc.), and acetic acid forming bacteria (AAB) (Santiago-Urbina *et al.*, 2013) that have been identified for their probiotic potentials. Since, yeast, LAB, Probiotics, and organic acids are used as growth-promoting additives in poultry feeding. Aged-palm wine could be exploited to enhance the performance of poultry, since it contains appreciable levels of these beneficial organisms, and organic substances.

2.13.1 Sources and microbial characteristics of palm wine

The palm sap contains a diverse collection of microbial community largely populated with yeasts, LAB, and AAB among other microbials (Ezeagu *et al.,* 2003). These organisms were reported to originally come from the indigenous flora resident in the inflorescence, and xylem stream of the palm trees (Okafor, 1972b; Faparusi, 1973) or introduced into the wine by the activities of invading insects, and from the tapper through his implements (tapping knives, funnels, and collecting gourds) during wine tapping (Nwaiwu *et al.,* 2016; Faparusi, 1973; Okafor, 1972a). Their presence in the sap plays a fundamental role in wine fermentation by initiating rapid degradation of its rich sugar reserves into several metabolites which significantly alters the physico – chemical constituents, and microbial composition of the wine produced.

The presence of yeast in palm sap for example, is reported to be responsible for the hydrolysis of sugars to ethanol, and volatile compounds (Ezeagu *et al.,* 2003; Okafor, 1978). However, of all the yeast species present in palm sap, *Saccharomyces cerevisiae* was identified as the most dominant yeast specie in palm wine (Stringini, Comitini, Taccari, and Ciani, 2009; Nwaiwu *et al.,* 2016; Karamoko *et al.,* 2016; Djeni *et al.*, 2020), and was implicated to be responsible for alcoholic content of palm wine. Authors have also identified *Saccharomyces chevalieri* as the dominant yeast specie in Toddy drink brewed from coconut palm wine in Asia, and Latin America. The activity of this yeast together with *Zymomonas mobilis* significantly increases the alcoholic concentration of fermenting wine (Obire, 2005; Alcántara-Hernández *et al.,* 2010). Other genera of non-*Saccharomyces* yeast such as *Pichia, Candida, Hanseniaspora, Saccharomycopsis, Saccharomycodes,* and *Endomycopsis* (table 2.1) have all been isolated from palm wine (Ouoba *et al.,* 2012; Amoikon, Aké, Djéni, Grondin, and Casaregola, 2018; Djeni *et al.,* 2020).

Similarly, a wide range of LAB, AAB and non-yeast organisms have been isolated from different palm wine sources at various stages of fermentation. Within the bacteria phylum, reports have identified *Lactobacillaceae, Leuconostocaceae, Acetobacteraceae, Sphingomonadaceae,* and *Enterobacteriaceae* (Table 2.1) to predominate wines produced from different palm species (Nwaiwu *et al.*, 2016; Karamoko *et al.,* 2016; Yang, Dou, and Ma, 2018) with LABs (*Lactobacillus*) dominating the others (Amoa-Awua *et al.,* 2007; Ziadi, M'hir, Kbaier, Hamdi, and Ferchichi, 2011). Recently, Djeni *et al.* (2020) identified LAB's (*Lactobacillus* and *Leuconostoc*), AAB's (*Acetobacter, Gluconoacetobacter* and *Gluconobacter*), *Sphingomonas* and *Enterobacteria* spps to predominate wine samples of oil palm, Raphia palm, and Ron palm produced in Cote d'Ivoire, while *Zymomonas* along with *Lactobacillus, Leuconostoc,* and *Acetobacter spps* predominated palm wine from Nigeria (Diaz *et al.*, 2019).

The diversity, and succession of these live organisms in palm wine would be dependent on several factors several of which include, the palm tree species, the composition of the sap, stage of fermentation, and certain conditions such as seasonality, plant physiology, age, soil conditions, and other environmental factors (Santiago- Urbina, and Ruiz-Teran, 2014; Fonseca-García *et al.,* 2016). Earlier studies by Okafor (1975), observed that *Serratia,* and *Brevibacterium* spps were found present in *Elaeis* wine but were absent in other wine sources, while *Sarcina,* and *Zymomonas spps* have been found only in Raphia wine. There seems to be more *Lactobacillus spp* bacteria in Ron and *Elaeis* palm wine than could be found in Raphia wines. According to Djeni *et al.* (2020), *Lactobacillus johnsonii,* and *Lactobacillus helveticus* (Table 2.1) are more abundant in Ron palm, and Oil palm saps compared to other palm wine sources, whereas *Leuconostoc mesenteroides* was abundantly present in *Raphia hookeri*. Coconut palm wine (Tuba drink) from Mexico is heavily dominated by *Fructobacillus,* and *Gluconacetobacter* compared to other LAB and acetic acid bacteria (Astudillo-Melgar *et al.*,

Table 2.1: Microbial content of palm wine

Source of palm sap	Microbial diversity	Examples	References
Raphia Palm wine	Yeast	*Saccharomyces cerevisiae, Schizosaccharomyces pombe, Saccharomyces uvarum, Candida spp.*	Obi *et al.,* 2015.
		Sacchoromyces cerevisiae, Pichia kudriavzevii, Candida tropicalis, Candida ethanolica.	Nwaiwu *et al.,* 2016
		Hanseniaspora valbyensis, Saccharomycodes, Saccharomyces,	Djeni *et al.,* 2020
		Pichia spp., Saccharomycopsis spp., Endomycopsis spp., Candida spp., Rhodotorula spp., Kluyveromyces marxianus, and Torulopsis spp. (*Candida glabrata*), *Saccharomycodes, Kleockera, Schizosaccharomyces*.	Trevanich *et al.,* 2016.
	LAB	*Lactobacillus spp., Leuconostoc spp., fructobacillus spp.*	Uzochukwu *et al.,* 1994; Djeni *et al.,* 2020.
		Lactobacillus plantarum, Lactobacillus lactis, Leuconostoc mesenteroides.	Akinrotoye, 2014
		Lactobacillus spp., Bacillus spp., Streptococcus spp.,	Obi *et al.,* 2015; Obi and Ekezuom, 2018.
	AAB	*Acetobacter syzygii, gluconacetobacter, gluconobacter*	Djeni *et al.,* 2020.
	Others	*Proteus vulgaricus, Pseudomonas aeruginosa, Micrococcus luteus*	Akinrotoye, 2014
		Staphylococcus aureus, E. coli, Micrococcus luteus, Serratia marcessens	Obi *et al.,* 2015
		Zymomonas, Sarcina	Djeni *et al.,* 2020
Oil *(Elaeis guineensis)* palm wine	Yeast	*Saccharomyces spp., Saccharomycoides spp., Schizosaccharomyces spp., Pichia spp., Micrococcus spp., Hanseniaspora guilliermondii, Hanseniaspora uvarum, Endomycopsis spp., Candida spp.,*	Djeni *et al.,* 2020
		Saccharomyces cerevisiae, Kloeckera apiculata (*Hanseniaspora uvarum*), *Candida krusei, Candida ethanolica, Candida tropicalis, Pichia kudriavzevii.*	Amoa-Awua *et al.,* 2007; Nwaiwu *et al.,* 2016

	LAB	*Lactobacillus spp., Leuconostoc mesenteroides, Bacillus spp., Streptococcus, fructobacillus spp*	Amoa-Awua *et al.*, 2007; Okolie *et al.*, 2013; Djeni *et al.*, 2020; Okafor, 1972.
	AAB	*Acetobacter, Gluconobacter, Gluconacetobacter*	Amoa-Awua *et al.*, 2007; Okolie *et al.*, 2013; Djeni *et al.*, 2020.
	Others	*Brevibacterium, Zymomonas, Micrococcus, Kluyvera, Serratia, Aerobacter, Pseudomonas, Cornybacterium,*	Okafor, 1972; Djeni *et al.*, 2020
Cocos nucifera palm wine	Yeast	*Saccharomyces chivalieri, Candida pelliculosa, Candida utillis, Stephanoascus ciferrii, Kloeckera spp, Trichosporon asahii, Rhodotorula mucilaginosa*.	Santiago-urbina, and Ruiz- Terran, 2014; Kadere *et al.*, 2014
	LAB	*fructobacillus, leuconostoc, Lactobacillus, Lactococcus*.	Kadere, and Kutima, 2012; Astudillo-Melgar *et al.*, 2019; Diaz *et al.*, 2019.
	AAB	*Acetobacter, Gluconacetobacter.*	Astudillo-Melgar *et al.*, 2019
	Others	*Vibrio, Sphingomonas, Erwinia, Klebsiella, Serratia, Cronobacter*	Astudillo-Melgar *et al.*, 2019
Ron (*Borassus spp*) palm wine	Yeast	*Saccharomyces cerevisiae, Hanseniaspora spp, Hanseniaspora guilliermondii*	Djeni *et al.*, 2020
	LAB	*Lactobacillus johnsonii, Lactobacillus helveticus, leuconostoc spp., fructobacillus spp., Lactobacillus gasseri,*	Djeni *et al.*, 2020
	AAB	*Acetobacter spp., gluconacetobacter syzygii,*	Djeni *et al.*, 2020
	Others	*Zymomonas,*	Djeni *et al.*, 2020.

Source: Compiled by the author.

2019; Diaz *et al.*, 2019). *Acetobacter indonesiensis* was reported as the predominant AAB in 'Bandji' palm wine of *Borassus akeassii* (Ron palm) from Burkina Faso (Ouoba *et al.,* 2012).

Wine fermentation could also modify bacteria succession in wine as the stages progress. For instance, Stringini *et al.* (2009) reported *Saccharomycodes ludwigii* as the dominant yeast strain at the beginning of palm wine fermentation but was overtaken by *Saccharomyces cerevisiae* after 24 hours, and completely became absent in the wine after 5 days of fermentation.

A study by Obi, Ogbulie, and Nkwo (2015) isolated 4 yeast and 8 bacteria species from a freshly tapped palm. While studying the succession of micro-organism with increased fermentation during storage, they observed the disappearance of *Staphylococcus aureus* after 24 hours, and the absence of *E. coli, Micrococcus luteus*, *Candida spp*, *Streptococcus spp* and *Lactobacillus spp* after 2 days of fermentation. The complete disappearance of *Serratia marcessens* was after 3 days, while *Bacillus spp*, *Acetobacter spp* and yeast (*Saccharomyces cerevisiae, Saccharomyces uvarum, Schizosaccharomyces pombe*) remained in the fermented palm wine till after 5 days. Similar studies by Obi, and Ekezuom (2018) also found *Bacillus spp*, *Lactobacillus spp,* and *Saccharomyces spp* as lone survivors in palm wine with increased fermentation process till 14 days while other organisms (*Staphylococcus aureus, Streptococcus spp* and *Micrococcus spp*, *E. coli* and *Candida spp* seized to exist after 5 days. Earlier reports by Okafor (1975) also showed the disappearance of *Serratia,* and *Aerobacter spps* from fermenting palm wine after about three days while, Astidullo *et al.* (2019) reported a decrease in population of *Vibrio*, and an increase of lactic acid bacteria (LABs), acetic acid bacteria (AABs) and, *Sphingomonas* with increased fermentation of the local wine (Tuba) brewed from coconut (*Cocos nucifera*) tree sap.

The gradual decrease or complete elimination of these microbes (mostly pathogenic) with progressive fermentation was associated with the increased acidification and decreasing pH of the fermenting palm wine resulting from the activities of LABs and AAB spps (Amoa-Awua *et al.*, 2007; Bassir, 1968). *Acetobacter* bacteria were more pronounced in palm wine after the third day of fermentation, and at which time substantial quantity of alcohol would have been produced as an appropriate substrate for acetic acid production by AAB (Okolie, Opara, Emerenini, and Uzochukwu, 2013; Okafor, 1975). Thus, the microbial diversity of aged palm wine of at least up to five days of fermentation seem to be more suited for use as a mixed probiotic population for promoting production performance of poultry.

2.13.2. Chemical characteristics of palm wine

Freshly tapped palm wine sap is a clear, colourless, and sweet syrup largely composed of sugars, predominantly sucrose, glucose, fructose, and maltose sugars (Ben Thabet *et al.*, 2009; Santiago-Urbina *et al.*, 2013), with a near neutral (7 – 7.4) pH (Ezeagu *et al.*, 2003, Lasekan, Buettner and Christlbauer, 2007), and various concentrations of alcohol, organic acids, proteins, minerals, and vitamins (Ogbonna *et al*., 2013; Karamoko *et al.*, 2016). According to reports (Faparusi, 1981, cited by Santiago-Urbina and Ruiz-Teran, 2014), sucrose is the most abundant sugar in palm sap. It also contains traces of other sugar molecules such as xylose, cellobiose, galacturonic acid, arabinose, lactose, and rhamnose etc.

(a) Alcohols: As the palm sap ages, it however undergoes natural fermentation, which promotes the proliferation of yeasts, lactic acid forming bacteria (LAB), and acetic acid forming bacteria (AAB) whose activities have been identified to significantly alter the biochemical, and microbiological properties of the sap. Firstly, the rich sugars are rapidly broken down into several metabolites (Table 2.2), mainly ethanol, lactic, and acetic acids (Amoa-Awua *et al*., 2007; Santiago-Urbina *et al*., 2013), thereby increasing the acidity of the palm sap with the consequent pH reductions (4.5 – 2.8) that follows (Amoa-Awua *et al.*, 2007; Astidullo-Melgar *et al.*, 2019). At this stage, the alcoholic content of the sap would have increased exponentially, especially when fermented for 5 – 7 days and, after which it declines due to the build-up of acetic acid (Amoa-Awua *et al.,* 2007; Santiago-Urbina and Ruiz-Teran, 2014). The AAB utilizes alcohol as a substrate for acetic acid production.

(b) Organic acids: Lactic acid has been reported as the main acid responsible for the acidification of aged-palm wine as it undergoes fermentative organic acid production (Ouoba *et al.,* 2012; Santiago-Urbina *et al.,* 2013; Karamoko *et al.,* 2016). Acetic, and tartaric acids have also been implicated to increase the acidity of the palm wine during prolonged fermentation. Traces of other organic acids such as oxalic, citric, malic, fumaric, pyruvic acid, succinic acid, and ascorbic acids (Table 2.2) have also been found in fermented palm wine (Karamoko *et al.,* 2012; Van Pee and Swings, 1971). These acids have been recognized for their inhibitory effects on pathogenic organisms especially on the members of *Enterobacteriaceae* (Hassan *et al.*, 2010; Hajati, 2018).

(c) Colourants, odorants and flavourants: Another consequence of the sustained fermentative process in palm wine is the dramatic change in colour, gas evolution and the formation of various flavour components that affects its organoleptic properties (Ojimelukwe, 2001; Obahiagbon and Oviasoge, 2007; Faparusi and Bassir, 1972). Volatile compounds such as esters, carboxylic acids, fatty acids, acyloin, etc., (Table 2.2) are reportedly contained in fermented palm wine thereby conferring the characteristic aroma, and taste of the wine (Uzochukwu, Balogh, Tucknot, Lewis, and Ngoddy, 1994; Nwaiwu *et al.,* 2016). Esters are however the dominant compounds among the group. A good example is the ethyl acetate reported for its dominant aroma (Nwaiwu *et al.,* 2016), while ethyl lactate influence more of palm wine flavour (Uzochukwu *et al.,* 1994). Ethyl acetate is formed by the combination of ethanol, and acetic acid during fermentation in palm wine (Uzochukwu *et al.,* 1994; Sumby, Grbin and Jiranek, 2010). Other potent odorants in palm wine include the earthy-smelling 3-isobutyl-2-methoxypyrazine, buttery-smelling acetoin, ethyl lactate, fruity ethyl-hexanoate, 3-

methyl-butyl acetate, butyl acetate, the popcorn-smelling 2-acetyl-1-pyrroline, pear-odour propyl acetate, strawberry methyl cinnamate, and potato-like butanol etc. (Lasekan *et al.,* 2007; Nwaiwu *et al.,* 2016).

(d) Proteins and amino acids: The protein, and amino acids profile of palm sap have also been reportedly affected as fermentation progressed from 0 - 7 days (Okafor, 1978). Both essential, and non-essential amino acids such as methionine, lysine, tryptophane, leucine, isoleucine, arginine, phenylalanine, cystine, aspartic acid, glutamic acid, serine, asparagine, citrulline, alanine, tyrosine, amino-butyric acid, valine, glycine, and proline (Table 2.2) have all been identified in palm sap, and fermented wine (Ibegbulem *et al.,* 2013; Bassir, 1968; Van Pee and Swings, 1971). As the wine fermentation progresses, cystine, cysteic acid, histidine, lysine, and arginine which were originally absent in freshly tapped palm sap, were respectively found after 12, 24, 36, 48, and 72 hours of fermentation, while threonine, ornithine, and histamine which were though present within the earliest stages of fermentation, disappeared from the wine after 24 hours (Bassir, 1968; Van Pee, and Swings, 1971). Faparusi and Bassir, (1972) reported a steady increase in total nitrogen content of palm wine with progressive fermentation till day 7.

(e) Vitamins and minerals: Palm sap have also been reported to contain some levels of macro, and micro mineral elements (Table 2.2) as well as essential vitamins (Ezeagu *et al.,* 2003; Ben Thabet *et al.,* 2009; Ape, John, Nwafor, and Ekpe, 2015). Ezeagu *et al.* (2003) reported that magnesium, and phosphorus were the chief minerals present in oil palm sap with some traces of lead, cadmium, and cobalt. Copper, iron, zinc, manganese, and calcium were also present in lower concentrations. Ben Thabet *et al.* (2009) reported potassium as the most abundant mineral fraction in *Phoenix dactylifera* palm sap, followed by magnesium, and phosphorus. The authors also recorded other minerals fractions in the sap in a decreasing order of concentration such as Ca, Na, Fe, Cu, and Zn.

Table 2.2: Chemical contents of palm wine during storage

Palm wine source	Products	Examples	References
Raphia palm	Sugars	Sucrose, Glucose, Fructose, Maltose, Xylose, Rhamnose, Arabinose, Cellobiose	Santiago-Urbina, and Ruiz-Teran, 2014; Faparusi, 1981; Faparusi, and Bassir, 1972.
		5-keto-D-fructose, ethyl-1-thio-alpha-D-arabinofuranoside, D-methyl-1-fucose)	Erukainure *et al.*, 2019
	Alcohol	Ethanol, Butanol, 1-propanol, Isopentyl alcohol, 3-methyl butanol, 2-phenyl ethanol.	Nwaiwu *et al.*, 2016
		Glycerine, 1-deoxy-D-arabitol	Erukainure *et al.*, 2019
	Organic acids	Lactic acid, Acetic acid, Propanoic, Hexanoic, Oleic	Nwaiwu *et al.*, 2016
		Pentanoic acid, Nonadecanoic acid, Dodecanoic, Tetra decanoic, Quinic, Palmitoleic acid, Octadecanoic acid, 2-hydroxy octanoic acid	Erukainure *et al.*, 2019
	Esters	Methylpropy lactate, Ethyl acetate, Methyl cinnamate, Ethyl hexanoate, Ethyl lactate, Methyl propyl acetate, Methyl-2-methyl propanoate, Ethyl dodecanoate, Ethyl octanoate.	Nwaiwu *et al.*, 2016
	Protein and amino acids	Methionine, Lysine, Tryptophane, Leucine, Isoleucine, Arginine, Phenylalanine, Cystine, Aspartic acid, Glutamic acid, Serine, Asparagine, Citrulline, Alanine, Tyrosine, Amino-butyric acid, Valine, Glycine, Proline.	Ibegbulem *et al.*, 2013; Bassir, 1968; Van Pee, and Swings, 1971.
	Minerals and vitamins	Mg, P, Cu, Fe, Mn, Pb, Cd, Co, Zn and Ca.	(Ezeagu *et al.*, 2003; Ogbonna *et al.*, 2013)
		Vitamin A, C.	Ogbonna *et al.*, 2013
Elaeis palm wine	Sugars	Sucrose, Fructose, Glucose, Raffinose, Maltose, Lactose, Arabinose, Xylose	(Ezeagu *et al.*, 2003; Nwaiwu *et al.*, 2016)
	Organic acids	Lactic acid, Acetic acid	Amoa-Awua *et al.*, 2007; Ouoba *et al.*, 2012; Santiago-Urbina *et al.*, 2013; Faparusi, 1981

		Lactic acid, Acetic acid, Tartaric, Oxalic, Citric, Malic, Fumaric, Pyruvic acid, Succinic acid, and Ascorbic acids	(Karamoko *et al.,* 2012)
		Propionic acid, Hexanoic acid, Oleic acid, Acetic acid	(Nwaiwu *et al.,* 2016)
		Acetic acid, Iso-butanoic acid, 2-methyl butanoic acid, 3-methyl pentanoic acid, phenylacetic acid and pentanoic acid	(Lasekan *et al.,* 2007).
		Benzoic acid, Phenylacetic acid, Phenyl propionic acid, Acetic.	
	Alcohol and volatile compounds	Ethanol, 1-propanol, Isopentyl alcohol, butanol, 2-phenyl ethanol, 3-methyl butanol, 2-3-methyl butanol, Acetoin	Lasekan *et al.,* 2007; Nwaiwu *et al.,* 2016
	Esters	Ethyl-2-butanoate, a-butyl acetate, Ethyl 3-methyl butanoate, Diethyl succinate, Ethyl 4-hydroxybenzoate 2-phenethyl octanoate.	Uzochukwu *et al.,* 1999.
		Methyl butanoate, Diethyl succinate, Ethyl lactate. 3-isobutyl-2-methoxypyrazine, Acetoin, ethyl hexanoate, 3-methyl-butyl acetate and 2-acetyl-1-pyrroline, Ethyl pentanoate, Ethyl octanoate, Ethyl-n-hex decanoate, Ethyl-3-phenylpropionate, Linalool.	Uzochukwu *et al.,* 1994; 1999; Lasekan *et al.,* 2007; 2009).
		Ethyl acetate, Propyl acetate, 2-methyl propanoate, Ethyl dodecanoate, Ethyl octanoate, Methyl cinnamate, Butyl acetate, Methyl propy lactate, Ethyl hexanoate, Ethyl lactate.	Nwaiwu *et al.,* 2016
	Proteins and amino acids	Methionine, Lysine, Tryptophane, Leucine, Isoleucine, Arginine, Phenylalanine, Cystine, Aspartic acid, Glutamic acid, Serine, Asparagine, Citrulline, Alanine, Tyrosine, Amino-butyric acid, Valine, Glycine, Proline	Ibegbulem *et al.,* 2013; Bassir, 1968; Van Pee, and Swings, 1971.
	Minerals and vitamin	Mg, P, Cu, Fe, Mn, Pb, Cd, Co, Zn and Ca	
Ron palm (Borrasus spp)	Sugars	Sucrose, Glucose, Fructose	Naknean *et al.,* 2010.
		Galacturonate, UDP-D-xylose, UDP-L-arabinose, UDP-D-galacturonate, deoxy hexose sugars, Myo-inositol.	Djeni *et al.,* 2020

Coconut palm	Sugars	Sucrose, fructose, galactose, mannose, glucose	Shetty *et al.*, 2017.
		Sucrose, Fructose, Glucose	Astudillo-Melgar *et al.*, 2019.
Phoenix palm wine	Sugar	Sucrose, Glucose, Fructose, Myo-inositol	Ben Thabet *et al.*, 2009
	Mineral	K, Mg, P, Ca, Na, Fe, Cu, and Zn.	Ben Thabet *et al.*, 2009
	Polyphenols	Gallic acid	

Source: Compiled by the author

Essential vitamins such as B_1, B_2, B_6, and B_{12}, were shown to double in concentration over a 7-day period of fermentative process while vitamin C concentration declined within the initial 24 hours of sap fermentation and later became stable in concentration (Bassir, 1968; Okafor, 1978). The biochemical constituents of palm wine seem to vary with the specie of palm tree tapped (Ibegbulem *et al.*, 2013; Lucky, Cookey, and Ideriah, 2017), tapping process (frequency of tapping, length of storage and collection time) (Karamoko *et al.*, 2012), season, and the environment (Tapsoba, Legras, Savadogo, Dequin, and Traore, 2015).

Reports have shown for example, that sugar concentrations of the palm sap were much higher during the early tapping stages but gradually declined with progressive increase in tapping length and storage time due to the microbial fermentation of the sugars to produce alcohol (Karamoko *et al.*, 2012; Santiago-Urbina *et al.*, 2013). According to Okafor (1978), the pH, sucrose, and vitamin C concentrations in palm wine decreased with increased tapping length, while other constituents such as ethyl alcohol, titratable acidity, total nitrogen, and vitamins B_1, and B_2, increased with tapping length. It was also observed that wine samples from *Raphia hookeri* contained more glucose, sucrose, fructose, and maltose on the first day of tapping than xylose, cellobiose, galacturonic acid, arabinose and rhamnose sugars which were present in much lower concentrations as the tapping days progressed (Faparusi, 1981, cited by Santiago-Urbina and Ruiz-Teran, 2014). The alcohol content of palm wine during the rainy season was found to be much higher than in the dry season (Bassir, 1968) while wine collected during the day had less alcohol than that accumulated over the night.

2.13.3. Palm wine as a source of probiotics and organic acids in animal production

Palm wine from different sources has been widely studied for its potential benefits to man, and animals alike due to its rich nutrient content (sugars, amino acids and proteins, vitamins, minerals) and heavy concentration of live micro-organisms particularly yeast, LAB and AAB.

The role of these organisms in sugar fermentation, organic acid production, and probiotic properties have been well researched and documented.

Probiotics are non-antibiotic growth-promoting feed additives directly fed to animals in order to successfully colonize the gut, and maintain intestinal microbial balance by the selective exclusion of pathogenic organisms from attachment sites and nutrients in the GIT (FAO, 2016) or modification of the immune system (Bai *et al.,* 2013; Forte *et al.,* 2016). Organic acids are weak acids that partly dissociates into ions in aqueous solution, and traditionally used as feed additives to reduce bacteria contamination and proliferation in the gut. Therefore, supplementing them in feeds will improve growth performance through improved digestibility of nutrient, (Zhang, and Kim, 2014; Lei *et al.*, 2015), increased digestive enzyme activities, SCFA and vitamins (Liao, and Nyachoti, 2017; Sharifuzzaman, and Austin, 2017).

Saccharomyces cerevisiae particularly, has gained popularity as a probiotic, and growth promoting agent in ruminant animal production (Ghazanfar, Anjum, Azim, and Ahmed, 2015) as well as in poultry (Bai *et al.,* 2013; Ahiwe *et al.,* 2020).

The inclusion of LAB organisms in layer diets reportedly increased laying performance in chickens, and body weight gain in turkeys (Gadde, Kim, Oh, and Lillehoj, 2017). Similar reports of improved egg quality, and eggshell characteristics have been observed (Sharma, Joshi, and Sharma, 2012). Improved immune responses and protection against *Salmonella typhimurium* were also observed in newly hatched chicks fed *Lactobacillus plantarum* (Wang *et al.,* 2018).

Moreover, of all the direct fed-microbial products reported in literatures, the strains isolated from the GITs are the most popular and commonly used as probiotic-additives in animal production (Svetoch *et al.,* 2009; Kabir, 2009). However, the use of palm wine as a probiotic source for improved performance and liveability in animal production is being understudied. Microbial isolates from palm wine were found to improve the performance of broilers when added in their diets (Bohoua, 2008; Alade, 2018). Yeast isolates from palm wine had strong antimicrobial activities against important pathogens (Fossi *et al.,* 2015; Satyalakshmi *et al.,* 2018). A study by Ojo and Agboola (2019), observed that fermented palm wine from *Raphia vinifera,* and *Elaeis guineensis* had inhibitory effect against *Salmonella typhi* compared to known antibiotic drugs such as chloramphenicol, amoxycillin, gentamycin, and ciprofloxacin.

Similar inhibitory responses were also recorded using a 7 – day old fermented *Raphia* wine against diarrhoeagenic pathogens such as *Salmonella typhi*, *Shigella dysentariae, Staphylococcus aureus,* and *E. coli*, compared to known antibiotics (Akinrotoye, 2014). The observed responses may not be unconnected with the increased concentrations of metabolic products of the fermenting wine such as organic acids, alcohol, diacetyl, carbon dioxide, and antimicrobial substances (bacteriocin, reutericycline) which have been reported to have anti-microbial activity against pathogenic bacteria (Suskovic *et al.*, 2010; Fossi *et al.*, 2015; Liao, and Nyachoti, 2017). The production of organic acids by the LABs and AABs induces an unfavourable micro-environment, and pH reductions inimical to the survival of pathogenic bacteria within the gut system (Dittoe *et al.*, 2018).

The LABs have been investigated for their bacteriocins-producing ability (Todorov, Franco, and Wiid, 2014). Bacteriocins are antimicrobial substances produced by bacteria organisms whose activities selectively inhibit the proliferation of bacteria other than the producing strain (Dobson *et al.,* 2012). They are most popular for their bactericidal or bacteriostatic effect against food-borne pathogens (Cheikhyoussef *et al.*, 2009).

Bacteriocin-producing LAB species isolated from different ecological niches other than palm wine have been well studied (Neal-McKinney *et al.,* 2012; Adebayo, Afolabi, and Akintokun, 2014). However, recent studies have successfully demonstrated the production of bacteriocin from LAB isolates of palm wine origin with inhibitory effects against pathogens. Fossi, Goghomu, Tongwa, Ndjouenkeu, and Cho-Ngwa (2017) identified bacteriocins from 3 LAB strains (*Lactobacillus plantarum, L. rhamnosus and L. brevis*) isolated from palm wines (*Elaeis guineensis* and *Raphia sudanica*) with inhibitory effect against *Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes*. Tongwa *et al* (2019) isolated three LAB species such as *Lactobacillus lactis*, *Lactobacillus plantarum* and *Lactobacillus brevis* from *Raphia mambillensis* palm wine with inhibitory effect against important pathogenic organisms.

In-vitro studies using *Lactobacillus rhamnosus* (IS9) isolated from palm wine showed strong inhibition of the growth of several strains of *Salmonella enteritidis* (Fossi, Bonjah, and Ndjouenkeu, 2017). Nisin, and enterocin from *Lactobacillus lactis* and *Enterococcus faecium* contained in Tuba wine inhibited a wide range of pathogenic bacteria (de la Fuentes-Salcido *et al.*, 2015).

Besides the production of bacteriocins, some LAB strains are a good source of digestive enzymes required to catalyse bio-chemical reactions in living systems. Their production of beta-galactosidase enzyme in high amounts aids lactose digestion (Aysun and Candan, 2003). Obi (2018) also isolated lactic acid-producing bacteria from palm wine samples which had increased enzyme (amylase, proteolytic and lipase) activities at elevated temperatures and acidic pH conditions. Similar findings by Sulistiani (2018) also reported enzymatic activities (amylase, protease and phytase) by LAB strains isolated from *Borassus flabellifer* palm wine. LABs exhibiting enzymatic activities improved growth performance (Santoso, Tanake, Ohtani, and Sakaida, 2001; Sarker, Kim, Jang, Sharmin and Yang, 2016) and enhanced nutrient digestion especially in newly hatched chicks (Taheri, Moravej, Tabandeh, Zaghari, and Shivazad, 2009; Angelakis, 2017). Dietary LABs with bile salt hydrolase (BSH) properties reduced serum cholesterol through improved excretion of bile salts, increased production of bile salts from serum cholesterol or by decreasing the solubility of cholesterol, thereby reducing its uptake from the gut (Nguyen, Kang, and Lee, 2007). Therefore, palm wine can serve as a probiotic source to improve performance of farm animals.

2.14. Effects of Combining AC, Probiotics, and Organic Acids on Animal Performance

With the increased intensification of livestock production, the use of additives to improve the performance of animals has become a common practice in the industry. The beneficial effect of feeding organic acids (OA), probiotics, and or activated charcoal, singly or in combination as growth promoters in animal production has been well documented. Reports of increased weight gain, and better feed to gain ratio in broilers (Hedayati, Manafi, Yari, and Vafaei, 2013 Tasharofi *et al*., 2017) and pig (Huo *et al.*, 2016; Ahmed, Mun, Song, and Yang, 2018), have been associated with dietary supplementation of organic acids. In laying hens, the dietary feeding of organic acids also improved egg laying, and eggshell quality (Swiatkiewicz, Koreleski, and Arczewska, 2010; Rattanawut, Trairabeap, Karrila, Rodjan, and Theapparat, 2019).

The efficacy of feeding activated charcoal to enhance productive functions in animals through improved GIT functions has also been demonstrated (Sivilai *et al.*, 2018; Schmidt, *et al.*, 2019). The feeding of activated charcoal was shown to enhance broiler performance (Kana *et al.*, 2011; Jiya *et al.*, 2013; Evans *et al.*, 2017; Durunna *et al.*, 2018), and improved performance and eggshell quality in laying hens (Ayanwale *et al.*, 2006; Prasai *et al.*, 2017). Gerlach and Schmidt (2012) in their experiment however, discovered that the addition of bamboo charcoal with bamboo vinegar solution in chicken feed enhanced digestion, and FCR in birds. Bamboo vinegar is a liquid by-product from charcoal production chiefly composed of organic acids, especially acetic acid (Gerlach, and Schmidt 2012). Authors have also observed that a blend of activated charcoal and organic acid when incorporated in layer diets improved egg production (Yamauchi *et al.*, 2010; Rattanawut *et al.*, 2021), eggshell quality, and strength (Kim *et al.*, 2006; Rattanawut *et al.,* 2017; Elsahn, 2021), and intestinal absorptive functions in white leghorn chicken (Samanya and Yamauchi, 2002; Yamauchi *et al.,* 2010). Improved body weight gain was reported in Betong chicken when 1% bamboo charcoal and vinegar (BCV) mixture was included in their diet (Rattanawut, 2014), with significant improvement in the intestinal morphology of Aigamo ducks (Ruttanavut *et al.,* 2009).

Similar effects of improved performance, and feed efficiency were reported in pigs with supplemental charcoal and organic acid mixtures (Chu *et al.,* 2013). Van Chao *et al*. (2016), also observed the ability of activated charcoal, and wood vinegar blend to reduce the frequency of diarrhoea, and emission of hydrogen sulphide (H_2S) in the faeces of fattening pigs.

The ability of organic acid and activated charcoal blend to modify the GIT of chicken was demonstrated by Rattanawut (2014) who observed less shedding of pathogenic microorganisms (*E. coli* and *Salmonella*) in the faeces of chickens fed dietary activated charcoal and wood vinegar blends. In an *in-vitro* study conducted by Watarai and Tana (2005), *Salmonella enteritidis* was adsorbed more effectively by 'Nekka' – rich AC with vinegar in the diets compared with a probiotic product (*Enterococcus faecium*). Again, the increased inclusion of this product directly fed to calves also had increased absorptive efficacy against *Cryptosporidium parvum* oocyst and decreased diarrhoea and faecal excretion of oocyst in infected calves (Watarai, Tana, and Koiwa, 2008).

The animal performance effects witnessed during the dietary supplementation of activated charcoal and organic acid may have been attributed to the interactions of toxin binding effects, improved digestion, and GIT modifications by the acid-charcoal blend through the reduction of pathogenic bacteria proliferation as well as increase in beneficial microflora. Studies have suggested that charcoal could bind plant secondary metabolites (Mekbungwan *et al.,* 2008; Kana, Teguia, and Fomekong, 2012), improve digestion, and absorption of nutrient (Bakr, 2008; Majewska *et al.,* 2011), through the promotion of the growth of intestinal villi (Ruttanavut *et al.,* 2009).

The combined feeding of organic acids, and probiotics was found to be beneficial in broiler production (Agboola *et al.,* 2015; Youssef, Mostafa, and Abdel-Wahab, 2017). Allahdo *et al.* (2018) reported improved feed efficiency at the early stage of broiler development when probiotics, and vinegar were added in the diets and water of broilers respectively. In another broiler study, the feeding of organic acids, and probiotics lowered blood cholesterol, and low-density lipoprotein levels in adult broilers (Moghadam, Rezaei, Niknafs, and Sayyahzadeh, 2009), and significantly improved meat quality (Sarker *et al.,* 2016a). Bamboo vinegar/liquid probiotic mixture, though did not significantly improve growth performance, caused reductions in back fat thickness, pathogenic faecal *E. coli,* and faecal odorous gases emission (H_2S and NH_3) in fattening pigs (Sarker, Bostami, Kim, Ji, and Yang, 2016). Similar reductions in faecal pathogenic *E. coli,* and ammonia emission in fattening pigs were also reported by Ahmed *et al.* (2018). They also observed that the introduction of probiotics into the wood vinegar fed to the experimental pigs not only reduced feed intake, but also improved crude protein digestibility compared to the control, and those on antibiotic therapy. Therefore, the combination of acetic acid, and probiotic as dietary supplement would increase the solubility of nutrients, and increase nutrient absorption, while controlling the balance of intestinal microflora thereby enhancing productive performance.

2.15 Synopsis of literature

The Nigerian poultry industry has emerged the most dynamic and fastest growing animal husbandry subsector in recent years. Unfortunately, the productivity of the industry is still limited and unable to meet her domestic demands for poultry products due to the astronomical rise in the cost of strategic inputs, technical, and economic inefficiencies, and poor-quality feeds.

Studies have suggested alternative feed resources that are not in direct competition to man and the dietary inclusion of functional additives as possible solutions to the problems mentioned above. Activated charcoal (AC) and organic acids (OA) are among the numerous additives (enzymes, probiotics, prebiotics antibiotics, phytogenic additives, ash minerals, mould inhibitors etc.,) which when present in feeds modify its characteristics, intake, digestibility, and gastro-intestinal flora of the animal fed, as well as the characteristics of the final animal product.

AC is produced by the activation and carbonization of agricultural residues (ARs) under controlled oxygen environment and characterized with enormous absorptive and bacteriostatic properties, while supplying some essential minerals to the animals. OAs are chiefly used to protect feed from microbial, and fungal proliferation, inhibit pathogenic bacteria proliferation in the GIT, enhance mineral absorption, improve nutrient digestibility, and positively influence gut microbiota. The use of aged-palm sap as a probiotic source and its blend with activated charcoal was shown to evoke performance responses in animals through improved efficiency in nutrient uptake and utilization. Their ability to enhance gut microbiota and reduce faecal emission of noxious gases have also been reported.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Locations

The study was carried out at the Teaching and Research Farm of the Federal University of Technology Owerri (FUTO), Imo state, located within the Southeast agro – ecological zone of Nigeria. Activated charcoal product and experimental diets were analysed at the School of Agriculture and Agricultural Technology Laboratory, FUTO. The haematological, serum biochemical and hormonal profile analysis was carried out at the Everight Diagnostic Centre Owerri. Tissue section and histological slides preparation were done at the Histopathology Department, Federal Medical Centre, Owerri.

3.2 Study layout

The study was carried out in three phases. Study 1 involved the production of activated charcoal-palm sap (ACAPS) feed additive from a blend of pig dung, palm kernel shell and bamboo wood and treatment with aged palm wine. Study 2 involved the evaluation of the effects of ACAPS on layer performance and egg quality. In study 3, the effect of the ACAPS as a feed additive in broiler production were evaluated.

3.3 Study 1: Production of Activated Charcoal-Palm Sap (ACAPS) Feed Additive

3.3.1 Collection and preparation of agricultural residues and palm sap

a. Agricultural residue: Freshly voided pig dung was collected using a clean plastic container from a local piggery farm located at Umuokile Enyiogugu in Aboh Mbaise Local Government Area (LGA), Imo State. The dung was spread and dried on a clean slab till it became crispy to touch. The palm kernel shells (PKS) were collected from a local oil mill located at Umuokile Enyiogugu in the same LGA. The PKS were washed with clean water, sieved thoroughly to remove sand and other particles and sun – dried. The bamboo wood was fetched from the same location as the pig farm and cut into small chips to enhance its combustion. The materials were weighed, such that 40% pig dung, 30% palm kernel shell, and 30% bamboo wood were blended into the pot for the activated charcoal production.

b. Palm sap: The palm sap of Raphia palm was purchased fresh from a tapper at Aboh Mbaise LGA and left in a loosely capped opaque plastic container on a cement floor to ferment at room temperature for 96 hours to convert its sugar content to alcohol and vinegar by the actions of the yeasts and bacteria present in the palm sap. The aged palm wine so produced at the end of the fermentation period was stored in a plastic container, ready for use.

3.3.2 Production of activated charcoal palm sap (ACAPS) feed additive.

(a) Design and construction of carbonization equipment: A clay pot of about 5-litre size with its lid was set-up on a firewood furnace while awaiting its loading with the agro-waste materials to be carbonized (Gunamantha and Widana, 2018).

(b) Carbonization of the materials: The carbonization of the agro-materials for activated charcoal production (AC) was done using a modified pyrolysis technique as described by Gunamantha and Widana (2018) and Okoli (2020). This process began with obtaining the materials for carbonization, which in this case was a blend of pig dung/palm kernel shell/bamboo wood at the ratio of 4:3:3 respectively. The agro – waste samples were weighed using a weighing scale (Camry) and then placed into the clay pot of about 5-litre size for carbonization. The pot containing the sample materials was placed on the fire set-up and then covered with the lid to initiate anoxic combustion process. The materials were allowed to burn for about 3 – 6 hours at about 300 ^{o} C till no more smoke was being produced.

(c) Activation of the charcoal: The carbonization of the loaded agro-waste materials led to the production of a charcoal product which was activated by the application of water on the burning charcoal. The pot was removed from the fire and the resultant product was allowed to cool and later ground into powder. Thereafter the activated charcoal was stored in an airtight plastic container with lid to prevent it from absorbing water.

(**d) Determination of activated charcoal yield:** Before the start of carbonization, the blended materials were first weighed to ascertain its weight. At the end of the carbonization and activation processes, the resultant product was weighed again to ascertain its yield. The apparent yield (Yo) was then evaluated at this stage using the relation.

$Yo\ (\%) = [W2 / W1] \times 100$

Such that,

Yo = apparent yield, %

W1 = weight of blended material before carbonisation, g

W2 = weight of activated charcoal after carbonization, g

(d) Treatment with fermented palm sap: A measured quantity of the activated charcoal with a known moisture content was mixed with a desired volume of fermented palm sap in a 7:3 ratio for AC and palm sap respectively and the resultant mixture becomes ACAPS which was stored in an airtight container for onward usage. The ratio of AC to palm sap was used to decrease the dustiness of the charcoal powder without significantly increasing the moisture content of the final product.

The flow diagram for the entire process is depicted in figure II. the mixing of AC and palm wine was done just before its addition to the experimental diets.

3.3.3 Physicochemical characterization of activated charcoal (AC)

The physico-chemical characteristics of the AC evaluated include, the bulk density, water holding capacity, specific gravity, pH, carbon content, pore volume, and oil absorption capacity.

A. Physical characteristics

(a) Determination of Bulk density (BD) of the AC

The method described by Makinde and Sonaiya (2007) and modified by Omede (2010) was adopted. The BD of the AC sample was poured into a Pyrex glass funnel of known volume till it was levelled off to the brim. The initial weight of the empty funnel was subtracted from the weight of funnel and its content. The BD of the AC was determined as the weight of AC divided by the known volume of the funnel.

$$\text{Bulk density} = \frac{\text{Weight of sample}}{\text{Volume of funnel}}$$

(b) Determination of water holding capacity (WHC) of the AC

The filtration method described by Makinde and Sonaiya (2007) was adopted with slight modification (Omede, 2010). A sample of the AC was poured into the Pyrex glass funnel line with filter paper and levelled off to the brim without pressing. Another filter paper was placed on the top of the AC sample. The weight of the dry AC sample was obtained by subtracting the initial weight of the empty funnel from that of the loaded funnel. The funnel and its content were set-up under a burette allowing water dropping to pass through this known volume of AC and the burette was stopped at the first drop of water from the funnel. The volume of water absorbed by the AC sample was read-off from the burette and the weight of the wet AC measured. Subtracting the weight of funnel and its dry content from that of its wet content gave rise to the water holding capacity of the AC sample, which is the weight of water held by the activated charcoal to the weight of the dry activated charcoal.

WHC = W2 – W1 (g water/g dry charcoal)

W1 = weight of funnel and the dry AC content

W2 = weight of funnel and wet AC content

(c) Determination of Specific gravity (SG) of AC

The specific gravity of a substance is a comparison of the density of the substance relative to a standard value (e.g., density of water). Similar procedure employed for the determination of BD was repeated. The SG value of the charcoal sample was determined as the ratio of the AC bulk density value to the density of water.

(d) Oil absorption capacity.

Oil absorption capacity was obtained as the weight of oil absorbed by the AC sample. 2 g of the AC sample was measured out into a beaker, and liquid paraffin was added until the dry powder disappeared (Sunjin Chem. Co. Ltd).

$$\text{Oil absorption Capacity (cc/g)} = \frac{\text{Final volume of liquid paraffin absorbed (cc)}}{\text{Weight of Sample (g)}}$$

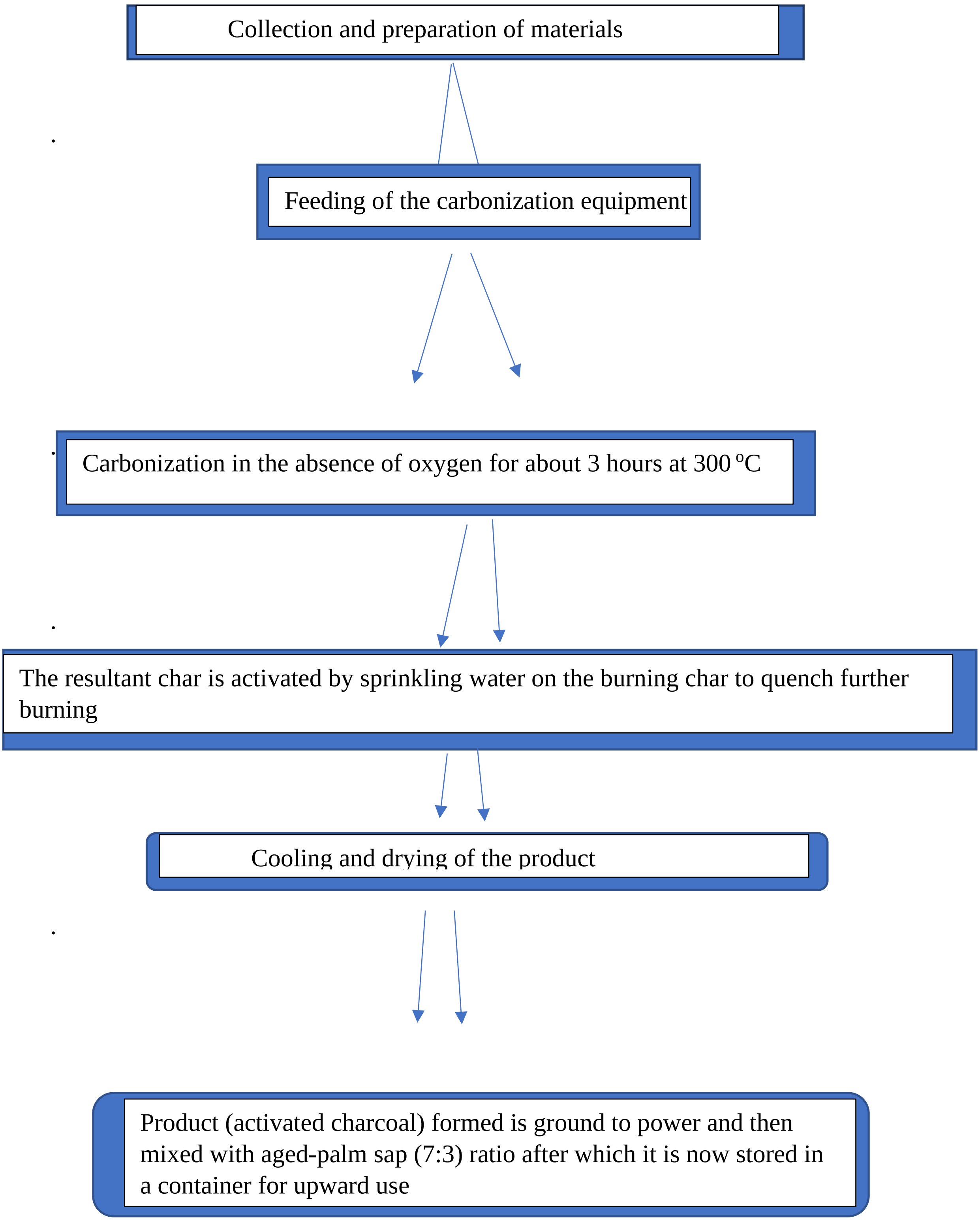

Fig. II: Flow diagram of ACAPS production

B. Chemical characteristics

a) Determination of pH of the AC

A known weight of the sample was dissolved in a known volume of deionized water and stirred to obtain a uniform mix. The pH of the mixture was thereafter determined using a hand-held pH meter (HANNA Combo pH Meter, Model: HI 98129).

b) Determination of the Carbon content of AC

A sample of the AC was collected in a ceramic crucible, placed in a furnace and heated till the sample became completely combusted and oxidised. The carbon content of the AC sample was obtained after subtracting the determined percentage values for moisture, ash, and volatile matter content from 100.

Equation: Fixed carbon (%) = 100 – (% Moisture + % Ash + % Volatile matter).

c) **Determination of Total Ash (TA):** Percentage ash determination was performed using the Carbolite model 1100 (AOAC, 2010). An empty crucible was placed in a desiccator and thereafter weighed to ascertain the weight of empty crucible or W_0. A measured weight of AC sample was placed in the crucible (W_1) and pyrolyzed in the furnace at 500 ± 15 ©C for 5-6 hours. The ashed sample was allowed to cool for about 30 minutes in the furnace and later placed back into the desiccator for cooling at room temperature for about 45 minutes. The crucible and its content were weighed again to determine its final weight (W_2).

Calculation**:** $\% \text{ Ash content} = \frac{(W_2 - W_0)}{W_1} \times 100$

d) **Determination of Volatile Matter (VM):** Adopting the total ash determination, the volatile matter content of the AC was determined as:

$$VM = \frac{W1-W2}{W1} \quad x\ 100$$

3.3.4 Analysis of the mineral content of activated charcoal

a. Sample digestion

A measured quantity (2 g) of the AC sample was weighed into a Kjeldahl flask; 20 ml of concentrated nitric acid (HNO_3) was added to the flask and thereafter pre – digested by heating gently for 20 minutes. More nitric acid was added to the digesta as heat treatment continued for about 30 – 40 minutes, till a clear digest was obtained. The Kjeldahl flask was cooled, before transferring its content into a 50 ml volumetric flask through a Whatman filter paper and thereafter made up to mark with distilled water. The resultant solution was analysed for mineral concentrations.

b. Determination of the mineral concentrations

The mineral concentrations of the AC material were determined using the Atomic Absorption Spectrophotometer (Spectrum Lab Model 23_A). Elements such as Calcium (Ca), Magnesium (Mg), Potassium (K), Phosphorus (P), sodium (Na), Manganese (Mn), Zinc (Zn), Copper (Cu), Iron (Fe), Chlorine (Cl) and Chromium (Cr) will be measured using the AAS.

3.3.5 Data Analysis

All analyses were done in triplicates and were subjected to ANOVA and mean separation using the SPSS analytical package (SPSS, 2012).

3.4. Study 2: Evaluation of the effect of ACAPS compound on performance of laying birds.

3.4.1. Experimental diets

The feed ingredients to be used in diet formulation were purchased from a reliable source in Owerri, Imo State. Five experimental diets were formulated such that the control diet (L1) had no activated charcoal-palm sap (ACAPS) compound supplementation. Another two diets (L2-L3) had graded levels of activated charcoal included in the diets at 0.50 and 1.00% of feed respectively and the last two diets (L4 – L5) had levels of ACAPS also included at 0.50 and 1.00% respectively as shown in table 3.1. All other ingredients were of equal proportions across the test diets.

Again, the proximate compositions and the physical characteristics of the experimental diets such as BD, WHC, SG and pH were determined as done in study 1. The mineral concentrations contained in the feed such as P, K, Ca, Mg, Na, Mn, Zn, Fe, Cu, and Cl were also determined as done in study 1. The diet electrolyte balance (dEB) of the experimental diets were calculated using the formula dEB = $Na^+ + K^+ - Cl^-$.

3.4.2 Experimental animals and design

One hundred and fifty (150) Isa brown layer birds of about 50 weeks of age were used in the experiment. The birds were divided into 5 groups of thirty birds per group, while each of the groups was further replicated three times with 10 birds per replicate in a completely randomized design (CRD). The birds were raised under the poultry management as practiced at the Teaching and Research Farm of FUTO.

At the beginning of the experiment, the birds were assigned to the treatment diets (L1 to L5) and fed for two weeks to acclimatize them before commencing data collection. Thereafter, the birds were provided with feed and water *ad libitum* throughout the duration of the experiment (twelve weeks).

Table 3.1: Nutrient composition of the AC and ACAPS based diet for laying birds

Ingredients g/100g	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS
Maize	43.00	43.00	43.00	43.00	43.00
Soya bean meal	18.00	18.00	18.00	18.00	18.00
Palm kernel cake	9.00	9.00	9.00	9.00	9.00
Fishmeal	1.00	1.00	1.00	1.00	1.00
Wheat offal	18.00	18.00	18.00	18.00	18.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
Limestone	6.00	6.00	6.00	6.00	6.00
Salt	0.25	0.25	0.25	0.25	0.25
Vit. /Mineral premix	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
*Activated charcoal	0.00	0.50	1.00	0.00	0.00
*ACAPS compound	0.00	0.00	0.00	0.50	1.00

2.5 kg premix Vitamin premix contains: Vit A = 10,000 000 I.U, Vit D3 = 2,000,000 IU, Vit E = 23,000 mg, Vit K_3 = 2000 mg, Thiamine (B_1) = 3000 mg, Vit. B_2 6000 mg, Niacin = 50,000 mg, Calcium Pantothenate = 10,000 mg, Vit. B_6 4000 mg, Vit B_{12} = 25 mg, folic acid = 1000 mg, Biotin = 50 mg, Choline Chloride = 400,000 mg, Manganese = 120,000 mg, Iron = 100,000 mg, Zinc 80,000 mg, Copper = 8500 mg, Iodine = 1500 mg, Cobalt = 300 mg, Selenium = 120 mg, Antioxidant 120 mg.

3.4.3 Data collection on production performance

a) **Feed intake:** Daily feed intake of each treatment group were determined each day by subtracting the weight of the left-over feed from the weight of the feed initially offered.

b) **Weight change:** The initial body weights of the birds in each replicate were taken at the beginning of the experiment and subsequently on a weekly basis around 7.00-9.00 am in the morning hours. Weight change was calculated weekly by subtracting the weekly weight measurement from the initial weight recorded.

c) **Hen-day production:** Eggs laid by each replicate group of birds were collected twice daily, morning (7 – 11 am) and evening hours (4 – 5 pm). The number of eggs collected each day were used to calculate the hen – day production according to the formula

Hen – day egg production (%)

$$= \frac{\text{Total No. of eggs laid/day}}{\text{No of Birds alive}} \times \frac{100}{1}$$

d) **Egg conversion ratio**: The egg conversion ratio was determined by calculating the quantity of feed required to produce 1kg of egg.

e) **Average egg weight**: All eggs collected from each replicate were weighed with an electronic digital scale to determine average egg weight and this was done weekly.

f) **Egg mass:** After weighing the representative samples of eggs to determine their average weights, egg mass was calculated by multiplying the average egg weight values with the hen-day production percentage values.

Average egg mass
(g/bird/day) = Hen-day production percentage x average egg weight (g)

3.4.4 Data collection on egg quality

A. Determination of external egg quality characteristics

a) Egg size:

- i. Egg length: Three eggs per replicate were selected for the determination of egg length using vernier callipers. Measurements were taken at the broad and narrow end of the eggs.
- ii. Egg width: Same eggs used for egg length determination, were also used for the determination of egg width using the vernier calliper which was placed at the broad cross-sectional region to obtain the diameter of the eggs.

b) Percentage shell: Three eggs selected at random per replicate were broken and the eggshells cleaned of dirt and dried of water, its membrane removed, and the shell will be weighed using an electronic digital scale. Percentage shell was determined by dividing the shell weight value with the egg weight and multiplied by 100.

c) Egg shape index: Egg shape index was determined as a ratio of the egg width to the length of the egg and multiplied by 100 using the following formula

$SI = (W/L) \times 100$, where W = egg width, L = egg length.

B. Evaluation of internal egg quality parameters

Three eggs per replicate were sampled randomly per week to assess the internal qualities of the eggs. The eggs sampled were weighed and broken individually on a clean flat glass surface for the determination of internal quality characteristics such as egg percentage components, eggshell thickness, egg yolk colour, albumen index, yolk index and Haugh unit.

a. Egg percentage components:

i. **Percentage yolk:** Selected eggs of known weight were broken into a flat glass surface, and the yolk separated from the albumin. The yolk was placed on a digital scale and the values read off and recorded. The percentage yolk was calculated as the ratio of yolk weight to that of egg weight and multiplied by 100.

ii. **Percentage albumin:** The weight of the separated albumin from the yolk was calculated as the difference in values obtained by subtracting yolk and shell weight values from the egg weight. The percentage albumin was calculated as the ratio of albumin weight to egg weight and multiplied by 100.

iii. **Albumen and Yolk Heights:** These were measured at the widest expanse and midway between the yolk edge and the external edge of the thick albumen of the broken eggs using micrometre screw gauge and vernier callipers respectively to the nearest 0.01 cm.

b. Shell thickness: This was measured with the micrometre screw gauge. Three eggs were taken from each replicate for this measurement. Membranes from the shells were first removed before measuring the egg thickness from three different areas of each shell. The value of the shell thickness was taken as the average of the three measurements.

c. Yolk colour: The egg yolk colour was evaluated visually by means of the La Roche scale (DSM Yolk Colour Fan). The blades of the DSM YolkFan^{TM} were held directly above the egg yolk while positioning the egg between the tips of the blade. The blade was viewed vertically with the blade numbers facing down, while the yolk colour values were read and recorded.

d. Albumen index: This was determined by dividing albumen heights with its corresponding lengths and widths.

$AI = (AH/ (AL+ AW) \div 2) X 100$

Where, AH = albumen height, AL = length of albumen, while AW = albumen width.

e. **Yolk index:** This was determined as a ratio of yolk height to its widths.

$YI = (YH/YW) X 100$

Where YH = yolk height, and YW = yolk width.

f. **Haugh Unit:** Haugh unit was calculated by using $Hu=100 \log (H + 7.5-1.7 W^{0.37})$ (Haugh, 1937; Oluyemi and Roberts, 2007), where

H = Observed albumen height (mm)

W = Observed weight of egg (g)

3.4.5 Determination of Reproductive Organ Characteristics

a) Weight of intact reproductive organ: At the end of the experiment (12 weeks), a bird from each replicate of the various treatments was randomly selected, weighed and slaughtered after fasting them over night. The birds were weighed, slaughtered, and allowed to bleed cleanly before defeathering and evisceration, to expose the internal organs. The ovary, oviduct, total ovarian follicles, large yellow follicles, small white follicles were removed immediately, and their weights measured collectively. The weights were expressed as percentages of live weights of the birds.

b) Weight of ovary: Upon evisceration of each bird, the ovary with its follicles intact were removed and weighed on a weighing balance to ascertain its weight which was further expressed as a percentage of the live weight. The weights of these organs were expressed as the percentage of live weight.

c) Weight of oviduct: As described above, the oviduct of each bird was weighed, and the values expressed as a percentage of the live weight of the bird.

d) Number of follicles: This was determined by visibly counting the number of follicles containing yolk. They were grouped according to weights into large yellow and white follicles, small yellow and white follicles. The numbers counted were recorded against each bird eviscerated.

3.4.6 Determination of haematological characteristics

A: Blood collection and processing

The blood samples were collected from a bird per replicate at the end of the 12 weeks experiment. Blood was collected from the jugular vein as the birds were being slaughtered for reproductive organ evolution. About 7 ml of various blood samples were collected, and 2 ml transferred immediately into sterile plastic bottles containing EDTA (Ethylene - diamine tetra - acetic acid) for haematological tests. The remaining 5 ml was transferred into clean Bjour bottles without anti-coagulant for serum production. The bottles were properly labelled and transported to the laboratory in a cold box within one hour of collection. The haematological parameters measured include, haemoglobin concentration, red blood cell count, packed cell volume, mean cell haemoglobin concentration, mean cell volume, mean cell haemoglobin, white blood cell count and white blood cell differential counts.

(a) Red blood cell (RBC) count: This was determined using the improved Neubauer counting chamber method as described by Schalm *et al.* (1975). 0.02 ml well mixed anticoagulant blood sample was discharged into 5 ml of diluents and mixed thoroughly by gentle inversion. Using a fine Pasteur pipette, the counting chamber was filled, excess fluid outside the surrounding channel was carefully blotted out. The cells were allowed to settle down and then three rectangular columns were counted using 10X eye piece microscope.

Formula:

$$\text{Erythrocytes Count} = \frac{N \text{ x } DF \text{ x } 10^6}{A \text{ x } D} \text{ per litre}$$

Where N = No of RBC's counted, DF = dilution factor, A = the area of the chamber counted, D = depth of chamber.

(b) Determination of haemoglobin (Hb) concentration: This was determined using haemoglobin cyanide method. In this method, the haemoglobin in the blood was oxidized to haemoglobin cyanide by the action of potassium ferro cyanide. The haemoglobin concentration was finally determined as described by ICSH (1996). 5.0 ml drabkins solution was poured into a tube before adding 0.02 ml anti-coagulated blood. This was mixed and allowed to stand for 10 minutes at room temperature for full colour development. The absorbance of the test solution was read off after zeroing the spectrometer with Drabkins reagent blank solution. The absorbance and its corresponding Hb concentration were read from a calibration chart prepared from standards solution of haemoglobin cyanide (BOH) and result expressed in g/dl.

(c) Determination of packed cell volume (PCV): The blood sample was mixed by gently rocking the container. The Winthrop microhematocrit tube was filled with blood by capillary action up to two thirds of its length. The sample was then spurned by centrifugation for 5 minutes at 10,000 rpm using the micro-haematocrit centrifuge machine (RM-12C BL) and the PCV values read as a percentage using the designed scale reader.

(d) Other haematological indices: These were calculated according to the formula.

The Mean cell haemoglobin (MCH) was determined as follows:

MCH (pg) = Hb x 10(g/l)/ RBC x /L

The Mean cell volume (MCV) was determined as follows:

MCV (fl) = PCV (L/L) x 10 /L /RBC

The Mean cell haemoglobin concentration (MCHC) was calculated as follows:

MCHC (g/l) =Hb x 10^2 (g/l)/PCV (L/L)

(e) White blood cell and its differential counts: The leukocyte or white blood count was obtained using a haemocytometer with Natt and Hendricks diluent to obtain a 1:200 blood dilution. The number of leukocytes were estimated in accordance with method of Schalm *et al.* (1975). Into 0.38 ml WBC dilution fluid was added 0.02 ml well mixed anti-coagulated blood and mixed thoroughly by gentle inversion, and then allowed to stand for 5-10 minutes for colour development. The counting chamber was charged with the resulting mixture and allowed to stand for the white cells to settle, while counting the 4 corner squares of the chamber.

Formular: $$WBC = \frac{N \times DF \quad \times 10^6}{A \times D}$$

Where N = the number of cells counted; DF = Dilution factor; 10^6= to convert to cells per litre; A = the area of chamber counted; D = the depth of chamber.

White blood cells were differentiated into granulocytes (heterophils), lymphocytes, monocytes, eosinophils, and basophils with the aid of automated WBC differential machine (Abbot i-STAT 1300 portable blood analyser).

3.4.7 Determination of serum biochemical characteristics

(a) Serum production: The 5 ml of blood samples collected without EDTA was used to produce the serum for biochemical analysis. Blood sample was allowed to clot at room temperature and thereafter centrifuged at 2200 r.p.m for 15 minutes to separate serum from other cellular components to obtain clear (non-blood stained) serum for biochemical assays. The serum parameters analysed were the total serum protein, albumin, globulin, urea concentration, creatinine, cholesterol concentration, serum enzymes, electrolytes and reproductive hormones.

(b) Determination of total serum protein (TSP) concentration: This was done using the Biuret method as described by Kohn and Allen (1995). Five millilitres (5 ml) of Biuret reagent was measured into 3 test tubes labeled “a”, “b” and “c” respectively. 0.1 ml of serum was washed into the “a” tube; 0.1 ml of standard protein was washed into the “b” tube, while 0.1 ml of water was washed into the “c” tube. Each of the tubes was mixed and allowed to stand for 30 minutes after which the “a” and “b” tubes were read off against the “c” tube at 540 nm on a visible light spectrophotometer (UNICAM SERIES 969).

Calculation: Serum total proteins (gm/100 ml) = Au/As x 6

(c) Determination of serum albumin and globulin concentrations: Serum albumin was determined using the Bromocresol Green (BCG) method, as described by Peter *et al*. (1982). Four millilitres (4 ml) of buffered BCG solution was measured into 3 test tubes. 0.02 ml of serum was washed into the “a” tube; 0.02 ml of standard albumin was washed into the “b” tube, while 0.02 ml of water was measured into the “c” tube. The tubes were allowed to stand for 5 minutes after which the “a” and “b” tubes were read off against the “c” using a colorimeter

(Spectrum Lab Model 23_A) at 630 nm wavelength on a calibration graph and the absorbance recorded.

Calculation: Serum albumin (gm/100 ml) = “a”/ “b” x 4

Serum globulin concentration was thereafter determined by subtracting albumin values from the total protein values.

(d) Determination of urea concentration

Blood urea was determined calorimetrically using the Thiosemicarbazone method of Marsh *et al.* (1965). Urea reacts with Diacetyl Monoxime in acidic conditions at nearly 100 ^{0}C to give a red coloured product which was measured calorimetrically at 520 nm. Thiosemicarbazone and ferric ions were added to catalyse the reaction and increase the intensity of its colour.

Calculations: (test/standards x 100 = urea (mg/100 ml)

(e) Determination of serum glucose: The glucose value was determined using the glucose oxidase method. Glucose was oxidized by glucose oxidase to produce D- gluconic acid and hydrogen peroxide. The hydrogen peroxide was then coupled with 4-amino antipyrine and phenol substitute oxidatively, in the presence of the peroxidase enzyme to give off the quinonimine dye which can be measured photometrically.

(f) Determination of serum cholesterol: Total serum cholesterol was determined using the method described by Fredrickson *et al.* (1967). About 10µl of distilled water, standard, and serum sample were respectively pipetted into three different test tubes labelled “unknown”, “Standard” and “Blank” accordingly. In addition, 1000 µl of the reagent was added to all the three sets of test tubes and thoroughly mixed before they were incubated at 25 ^{o}C for 10 minutes. The absorbance of the sample was read against the “blank” within 60 minutes at wavelength of 500 nm to determine the cholesterol concentration.

(g) Determination of serum creatinine: This was determined using Jaffe’s alkaline picrate method as reported by Bronses and Taussky (1945). The complexing of creatinine with alkaline picrate reacted with picric acid to form a red tautomer of creatinine picrate, the intensity of which was measured using a colorimetric technique. A known volume (1 ml) of the appropriately diluted serum was added into a test tube, while 0.1 ml of the reagent was added. The reagent was added to 1 mL of distilled water in another test tube to constitute the blank. For the standard, 0.1 mL of reagent was added to 1 ml of the standard reagent in another test tube and thoroughly mixed. After 30 seconds, the absorbance of the standard and sample were read at 492 nm. Exactly 2 minutes later, absorbance of standard and sample were also read.

(h) Determination of serum enzymes: Serum enzymes such as alkaline phosphate (ALP) alanine amino transferase (ALT), and aspartate amino transferase (AST) were estimated using colorimetric methods as described by Bergmeyer and Bernt (1974). The amount of oxaloacetate or pyruvate produced by transamination reacted with 2, 4 dinitrophenyl hydrazine (DNPH) to form a brown-coloured hydrazone, the colour of which in alkaline solution was read at 520 nm.

0.5 ml of DNPH was added into test tubes containing sera and buffered substrate and incubated was incubated in a water bath. The whole mixtures were mixed and allowed to stand at room temperature. After 20 minutes, 0.5 ml of 0.4N NaOH was added to each test tube. The results were read off with a colorimeter at 520 nm after 5 minutes.

i) Determination of serum mineral concentrations

Minerals in blood Ca, P, K, Na, Mg, Fe, Cl and HO_3) were determined using atomic absorption spectrometry as earlier described. The ratios of the different minerals were calculated and related to the dietary electrolyte balance earlier determined.

3.4.8. Determination of reproductive hormones

The hormones determined were the follicle stimulating hormone (FSH), luteinizing hormone (LH), oestrogen, and progesterone. About 5 ml of blood samples without EDTA was collected and later allowed to completely coagulate at room temperature before centrifugation at 10000 relative centrifugal force (RCF) for 15 mins in a temperature-controlled centrifuge (Thermo Scientific Benchtop Centrifuge). The serum was stored at 2-8 °C in polyethylene tubes after which serum concentrations of these hormones were determined using Chemiluminescence immunoassay (CIA) analyser MAGLUMI series (Shenzhen New Industries Biomedical Engineering Co., Ltd., Shenzhen, China). The CIA procedures were conducted following the manufacturer’s protocol. The analyser automatically calculated the hormone concentrations of each sample by means of a 2-point calibration master curve procedure.

3.4.9. Determination of faecal quality and mineral uptake

At the 12 weeks of the experiment, faecal samples were collected from each treatment group and prepared for mineral content analysis. Bags were placed on the floor of each pen to collect faecal droppings of the birds. The samples were weighed fresh, then air dried until they became crispy to touch to determine their moisture contents. Thereafter, they were subjected to AAS analysis as earlier described to determine their concentrations for minerals. Nitrogen content was determined by Kjeldahl method. Differences between feed and faecal mineral gave an estimate of mineral uptake. The faecal characteristics of each treatment was determined visually by checking for litter colour, odour and fly population**.**

3.4.10 Economics of egg production

The economics of production was calculated to determine the cost of producing 1 kilogram of feed vis a vis the cost of producing a kilogram of egg. The cost of feed per kilogram weight of feed (feed cost /kg) was determined by dividing the cost of feed ingredients purchased by the quantity procured, while feed cost per kilogram of egg was calculated by multiplying the feed cost per kg of feed with the FCR of each treatment. i.e., Feed cost/Kg X FCR.

3.4.11 Data analysis

Data collected were subjected to analysis of variance and differences between the treatment means were compared using the Duncan's multiple range test using Statistical Package for Social Sciences (SPSS) User's Guide version 20.00 (SPSS, 2012).

3.5 Study 3: Determination of the Effects of Additive supplementation of ACAPS on the Performance of Broilers.

3.5.1 Preparation of experimental diets

The experimental broiler diets were prepared following the procedures adopted for the layer diets. In the experiment, two types of broiler diets namely starter and finisher diets were formulated. During the broiler starter and finisher phases, four experimental diets were formulated such that the control diets (B1) had no activated charcoal, B2 – B3 had activated charcoal only, supplemented at 0.5 and 1.00 %, whereas the other two diets (B4 – B5) at each phase had levels of ACAPS added diets at 0.5 and 1.00% of feed as shown in tables 3.2 and 3.3 respectively. All other ingredients were of equal proportions across the test diets.

Proximate composition of the experimental diets was determined at the laboratory. The physical characteristics of the experimental diets such as BD, WHC, SG and pH were determined as done in study 2, while the mineral concentrations of the experimental diets such as Ca, P, Na, Mg, K, Mn, Zn, Fe, Cu, and Cl were also determined as done in study 2. The diet electrolyte balance (DEB) of the experimental diets were calculated as done in study two.

3.5.2. Experimental Animals and Design

One hundred and fifty (150) Abor Acre broiler day old chicks were used in the experiment. The birds were divided into 5 groups of thirty birds and each of the groups was further replicated three times with 10 birds per replicate in a completely randomized design (CRD). The birds were raised under the management system as provided by the Teaching and Research Farm of FUTO.

3.5.3 Data collection

(a) Determination of growth performance: The initial live weights of the birds were recorded at the beginning of the experiment and subsequent weighing was done weekly on individual bases in the morning hours (7.00 – 9.00 am) till the end of the experiment. Weekly weight gain was obtained by subtracting the initial live weight from the current weekly live weight.

Feed intake was determined on a daily basis by subtracting the weight of the leftover feed from the weight of the feed initially offered. Feed conversion ratio (FCR) was calculated by dividing the total feed intake by final weight gain, while the feed efficiency was derived as the inverse of the FCR (Esonu, 2015).

Table 3.2: Nutrient composition of the AC and ACAPS based diet for starter broilers

Ingredients g/100g	B1 Control	B2 0.50 AC	B3 1.00 AC	B4 0.50 ACAPS	B5 1.00 ACAPS
Maize	52.00	52.00	52.00	52.00	52.00
Soya bean meal	17.00	17.00	17.00	17.00	17.00
Groundnut cake	16.00	16.00	16.00	16.00	16.00
Palm kernel cake	6.00	6.00	6.00	6.00	6.00
Fishmeal	4.00	4.00	4.00	4.00	4.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
Limestone	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30
Vit. / Mineral premix	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
*Activated charcoal	0.00	0.50	1.00	0.00	0.00
*ACAPS	0.00	0.00	0.00	0.50	1.00

Vitamin premix contains the following per kg of feed: Vit A = 12,500,000 IU, Vit D3 = 2,500,000 IU, Vit E = 40,000 mg, Vit K_3 = 2,000 mg, Thiamine (B_1) = 3,000 mg, Vit. B_2 5,500 mg, Niacin = 55,000 mg, Calcium Pantothenate = 11,500 mg, Vit. B_6 5,000 mg, Vit B_{12} = 25 mg, folic acid = 1,000 mg, Biotin = 80 mg, Choline Chloride = 500,000 mg, Manganese = 120,000 mg, Iron = 100,000 mg, Zinc 80,000 mg, Copper = 8,500 mg, Iodine = 1,500 mg, Cobalt = 300 mg, Selenium = 120 mg, Antioxidant 120 mg.

Table 3.3: Nutrient composition of the AC and ACAPS based diet for finisher broilers

Ingredients	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS
Maize	62.00	62.00	62.00	62.00	62.00
Soya bean meal	22.00	22.00	22.00	22.00	22.00
Groundnut cake	5.00	5.00	5.00	5.00	5.00
Wheat offal	2.00	2.00	2.00	2.00	2.00
Palm kernel cake	2.00	2.00	2.00	2.00	2.00
Fishmeal	2.00	2.00	2.00	2.0	2.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.0	1.00
Limestone	1.00	1.00	1.00	1.00	1.00
Salt	0.30	0.30	0.30	0.30	0.30
Vit. / Mineral premix	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00
*Activated charcoal	0.00	0.50	1.00	0.00	0.00
*ACAPS	0.00	0.00	0.00	0.50	1.00

Vitamin premix contains the following per kg of feed: Vit A = 12,500,000 IU, Vit D3 = 2,500,000 IU, Vit E = 40,000 mg, Vit K_3 = 2,000 mg, Thiamine (B_1) = 3,000 mg, Vit. B_2 5,500 mg, Niacin = 55,000 mg, Calcium Pantothenate = 11,500 mg, Vit. B_6 5,000 mg, Vit B_{12} = 25 mg, folic acid = 1000 mg, Biotin = 80 mg, Choline Chloride = 500,000 mg, Manganese = 120,000 mg, Iron = 100,000 mg, Zinc 80,000 mg, Copper = 8,500 mg, Iodine = 1,500 mg, Cobalt = 300 mg, Selenium = 120 mg, Antioxidant 120 mg.

These data were determined for the starter phase on the 7th day of life and on the 4th week of life, while for the finisher phase, the values were determined at the 6th, 7th, and 8th weeks of the experiment. At 7th week of life, faecal samples were collected from the birds and dried for determination of mineral uptake from the experimental feeds. Faecal moisture was also determined at 7th week of the experiment.

(b) Determination of seventh day performance parameters

(i) Determination of growth performance: On the seventh day of life, one bird each was taken from each replicate to determine their growth performance as described earlier. These birds were thereafter sacrificed and used to determine gastro-intestinal tract (GIT) and liver development as well as GIT content pH using the methods as described by Ohanaka (2016).

(ii) Determination of GIT development: The GIT of sacrificed birds was carefully dissected out according to the method described by Ukwu (2013) and Ohanaka (2016), with each segment such as the crop, proventriculus, gizzard, small intestine and large intestine tied to prevent mixing of content. Thereafter, the segments were sectioned at the tied positions and their contents emptied carefully into clean and labelled test tubes. The different segments without their digesta content and the liver were weighed using an electronic weighing balance (*LP* 402_{A}), and their weights expressed as percentages of the live weight.

(iii) Determination of GIT content pH: A measured quantity of each GIT content was weighed into a glass beaker and an equal amount of distilled water added to the sample. The content of the beaker was stirred to obtain a slurry solution and then covered with watch glass. The solution was allowed to stand for an hour, stirring every 10 to 15 minutes, to allow the pH of the GIT slurry to stabilize. After one hour, the sample temperature was measured, and the temperature controller of the pH meter adjusted to that of the sample temperature. This adjustment was done just prior to testing. Immediately before immersing the electrode into the sample, the sample was thoroughly stirred with a glass rod. The pH electrode was now placed into the GIT slurry solution. After 30 seconds, the pH meter was read, and values recorded.

(iv) Determination of Intestinal Villi Length and Crypt Depth: The tissue samples of the jejunal section of the small intestine were taken from a bird per replicate and were cut into several pieces measuring 1 mm x 1 cm/ piece and transferred into glass tubes containing formalin solution. Tissue samples were trimmed and fixed in Bouin fixative for 24 hours. They were further embedded in wax, sectioned at 5 - 6 µm with microtron (Heitz and Wetzouir) and stained with haematoxylin and eosin (H & E) according to King *et al*. (1981). The prepared slides were viewed under the light microscope (Leica DM750) and the villi measured to ascertain the lengths.

3.5.4 Determination of Carcass and Organ Weights Characteristics

(a) Carcass characteristic: At the end of the finisher phase of the experiment (08 weeks), a bird from each replicate of the various treatments was randomly selected, weighed, and slaughtered for the evaluation of carcass, and organ weight characteristics. The birds were defeathered and eviscerated. The dressed weight of the birds was measured and used to determine the percentage dressed weight. Thereafter, the carcasses were dissected, and cut in parts such as breast muscle, thigh, drumstick, wing, back, neck, head, and shank length. The weights of each of these cut parts were expressed as percentages of live weight of the bird.

(b) Broiler skin colour: The broiler skin colour was determined by visual observation using a colour-graduated visual aid colour fan, the DSM colour fan (RCF, DSM Switzerland), with minimum and maximum values ranging from 1 to 15. The DSM was used to measure pigmentation of the shank, breast, thigh, back skin, and abdominal fat during carcass characteristics evaluation. A chicken per replicate was selected at random, slaughtered and evaluated immediately. The colorimetric values for each part of the body to be tested was assigned to 3 individual readers. The values were averaged to obtain the mean colorimetric values for the treatments (Liu *et al.*, 2008).

(c) Organ weight: Organs of the broiler carcass such as the heart, gizzard, liver, lungs, kidneys, and intestines were weighed-out and expressed as percentages of the live weight of the birds. The intestinal lengths were also determined in centimetres.

3.5.5 Economics of meat production

This was calculated to determine the cost of producing 1 kilogram of feed vis a vis the cost of producing a kilogram of meat at 6th, 7th, and 8th weeks of feeding. The cost of feed per kilogram weight of feed (feed cost /kg) was determined by dividing the cost of purchased feed materials by the quantity procured, while the determination of cost of producing 1 kilogram of meat was calculated by multiplying the feed cost per kg of feed with the FCR of each treatment. i.e Feed cost/Kg X FCR.

3.5.6 Blood collection and determination of haematological parameters

The blood sample was collected from a bird per each replicate group at the end of the 8 weeks of experiment. About 7 ml of various blood samples were collected from the vein under the wing web and about 2 ml of the blood was transferred immediately into sterile plastic bottles containing EDTA (Ethylene - diamine tetra - acetic acid) to prevent clotting and were prepared for haematological tests, as earlier described in study 2.

3.5.7 Blood chemistry determination

The remaining 5 ml of blood samples earlier collected without EDTA were prepared for the serum biochemical determination as analysed in study 2.

3.5.8 Serum minerals

The serum minerals were determined using atomic absorption spectrometry as earlier described in study 2.

3.5.9 Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) and differences between the treatment means were compared using the Duncan's Multiple Range Test using the IBM Statistical Package for Social Sciences (SPSS) User's Guide, Version 20.00. (SPSS, 2012).

3.6 Work plan

The proposed work plan is presented in Figure iii. The study lasted for about 14 months made up of 3 months each of studies 1, 2 and 3 respectively and 6 months of analysis of results and report writing.

3.7 Budget estimate

The estimated budget for the study is outlined as follows

Study 1	N 95,000.00
Study 2	N 791,000.00
Study 3	N 535,000.00
Project management activities	N 150,000.00
Thesis writing and production	N 125, 000.00
Total	N 1,696,000.00

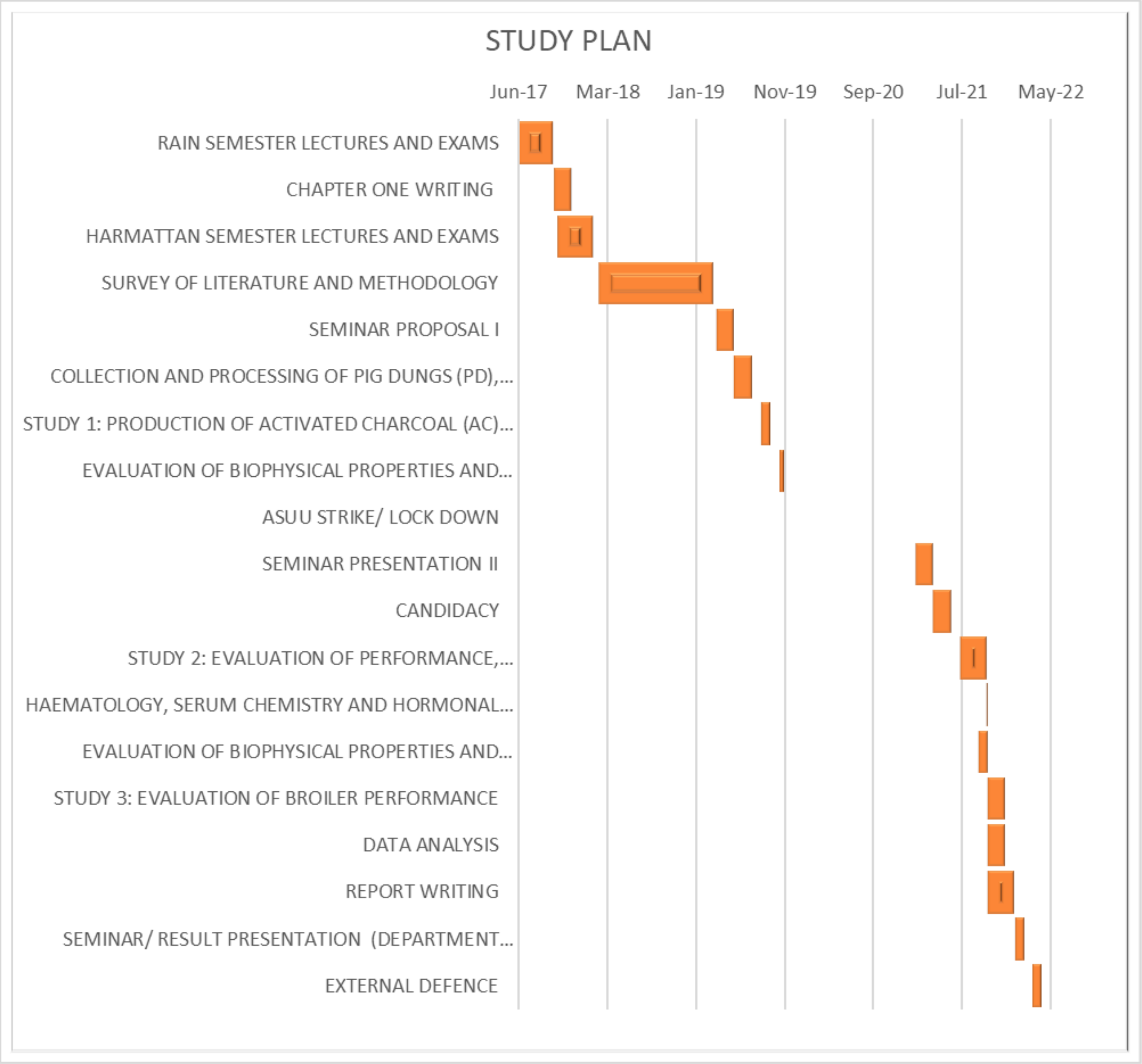

Fig. III. Gantt chart of the proposed work plan

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Activated charcoal production and physico-chemical characteristics

4.1.1 Yield and chemical characteristics of activated charcoal produced from biomass blends

The results in Table 4.1 showed that after pyrolysis, the blend of pig dung/bamboo stick/palm kernel shell yielded 46.54% activated charcoal. The AC yield is higher than the reported percentage yield for wood, and agro-residue derived activated charcoals (Mozammel *et al.,* 2002; Ma *et al.,* 2017).

It had a slightly alkaline pH value at 8.49, with a high carbon content (75.35%) and total ash content (13.13%). The pH value is within the range of 6 - 10 reported for most agro-residues (wood, and shells), and manure-derived ACs, especially those produced at high temperatures as well as some commercially available ACs (Yargicoglu, Sadasivam, Reddy, and Spokas, 2014; Chen, Qin, Sun, Cheng, and Shen, 2018). Activated charcoals are more effective as adsorbents at low pH than at high pH (Madu and Lagide, 2013). ACs having pH ranges of 6-8 are useful for most applications (Okeimmen, Okiemen, and Wuana, 2007), while lower pH ACs have more adsorption capacity compared to ACs with pH values of 12 (Auta and Hameed, 2013; Hadi, Xu, Ning, Sze Ki Lin, and McKay, 2015). The carbon content value recorded for the AC was 75.35%, while the total ash content value was 13.13%. These values are like the submissions of Mozammel, Masahiro, and SC, (2002) who reported 76.32, and 13.08% fixed carbon, and ash contents respectively. Lower percentage fixed carbon of 26.315 and 60.80% have been reported by Ma *et al.* (2017), and Cantrell *et al.* (2014) for PKS – derived AC, and a blend of poultry litter, and switch grass-derived AC respectively. Lower ash percentages (6.1 – 6.38%) have also been recorded for PKS-based AC (Evbuomwan *et al.*, 2013; Ma *et al.*, 2017), and bamboo AC (Yamauchi *et al.,* 2010),

Table 4.1: The yield and characteristics of AC produced from the biomass blends

Parameters	Values
Weight of biomass material (kg)	4.00
Loss in weight of biomass (kg)	2.14
Weight of activated charcoal	1.86
AC yield (%)	46.54
Carbon content (%)	75.35
Total ash (%)	13.13
Moisture content of AC (%)	11.54
Ph	8.49

while Samanya and Yamauchi (2001) found activated charcoal, and wood vinegar mixture to contain 13.40% ash. Higher ash content values (22 – 65%) were however reported for both manure, and wood-based ACs (Yargicoglu *et al.*, 2014; Cantrell *et al.*, 2014).

The variations in carbon, and ash contents of AC are influenced by factors like the pyrolytic temperature, activation process/agent, and nature of material among others. High pyrolytic temperature will yield ACs with higher ash content, and decreased yield (Yorgun and Yildiz, 2015; Tadda *et al.,* 2016). Again, the nature of the precursor biomass (pig dung, palm kernel shell and bamboo chips) like its lignin, cellulose, and hemicellulose contents influences the carbon content, ash, and pH of the AC produced (Ronsse, van Hecke, Dickinson, and Prins, 2012; Tomczyk, Sokolowska and Boguta, 2020).

4.1.2 Physical characteristics of the activated charcoal

Table 4.2 shows the physical characteristics of the AC produced from the biomass blends. Results obtained for the bulk density (BD), water holding capacity (WHC), and specific gravity evaluation of the AC produced in this study were 0.871 g/cm^3, 0.879 g water/g AC and 0.725 respectively. The BD was slightly higher than the 0.64 g/cm^3, and 0.73 g/cm^3 reported by Evbuomwan, Agbede, and Atuka (2013), and Yargicoglu *et al.* (2014) for PKS-derived and wood-derived ACs respectively.

Bryne and Nagle (1997) reported a range of 0.06 – 1.03 g/ cm3 for wood-based ACs. High BD signifies increased mechanical strength or hardiness of the resultant AC, which is a good attribute of AC required for water treatment/filtration processes (Zarifah, 2010). Adequate mechanical strength helps to reduce dust formation resulting from continuous friction between two AC particles (Vijayan *et al.*, 2012).

Table 4.2: Physical characteristics of Activated Charcoal

Parameter	Value
Bulk density (g/cm^3)	0.871
Water holding capacity	0.879
Specific gravity	0.725
Oil absorption capacity (g/g)	1.25

Bulk density, and WHC are the two bio-physical characteristics of powdered materials that could affect the nutritional value of animal feeds (Sundu *et al.,* 2008; Omede, 2010) since they have negative correlation with feed intake in animals. Besides, the BD, and WHC values of the AC in the present study also fell within the range reported for most conventional feedstuffs, and agro-waste derived ACs, and so may not negatively affect feed consumption when incorporated into poultry diets.

The specific gravity of a powdered material is the ratio of the density of the material to the density of water. Specific gravity of feed, and feedstuffs play important roles in the transit properties of digesta particles in the gastrointestinal tract (Omede, 2010). The SG value of 0.725 obtained in this study for AC was lower than the values (1.26 – 1.61) reported for shell-derived activated charcoal (Fono Tamo *et al.,* 2014; Boadu *et al.,* 2018). AC products with SG values less than 1 will traditionally float on water and thus, will have lower retention time in the gastrointestinal tract (GIT) of animals when ingested. The SG value of AC obtained in this study was however much higher than the range of 0.33 – 0.46 reported for conventional feed resources, and broiler rations produced in Nigeria (Omede, 2010; Omede *et al.,* 2011), indicating that it may impact diets after incorporation.

The AC produced from the blend of pig dung, PKS and bamboo sticks recorded oil adsorption capacity (OAC) value of 1.25 g/g meaning that 1 g of AC adsorbed 1.25 g of vegetable oil. OAC of a given sorbent is dependent on its surface chemistry, porosity, bulk density, affinity for oil, and the nature/type of oil sorbate to be adsorbed (Bandura *et al.,* 2017). The OAC value decreases with increased bulk density of a sorbent, while low BD favours the formation of capillaries which efficiently absorb oil within its pores (Angelova *et al.,* 2011). The OAC value of our AC product however lies within the range reported for most commercial sorbents and will efficiently adsorb oil from soil/water surface (Bandura *et al.,* 2015).

Table 4.3: Concentration of minerals in activated charcoal

Parameter	Result
Calcium (mg/kg)	5550.28
Phosphorus (mg/kg)	25100.66
Sodium (mg/kg)	1405.18
Magnesium (mg/kg)	4410.10
Potassium (mg/kg)	9300.29
Manganese (mg/kg)	671.33
Iron (mg/kg)	1304.65
Zinc (mg/kg)	103.48
Copper (mg/kg)	39.46
Chlorine (mg/kg)	25.46

4.1.3 Mineral composition of the activated charcoal

The mineral characteristic of the AC is summarized in table 4.3. The study showed that the major metals in the AC were phosphorus, potassium, calcium, magnesium, sodium, and iron with chlorine recording the least concentration. The order of mineral concentration was therefore P> K> Ca> Mg> Na> Fe> Mn> Zn> Cu> Cl. Several studies have found some level of mineral concentration in ACs produced from different agro-wastes, and residues. The concentration of minerals obtained in this study were much higher than the values reported for PKS-derived activated charcoal (Evbuomwan *et al.,* 2013), but lower than the values reported for wood, and coal derived activated charcoal (Machida, Yamazaki, Aikawa and Tatsumoto, 2005). The variations may be because of carbonization temperature which destroys the carbon structure, and as such, influences the level of ash produced during AC production. Again, some feedstocks containing high volatile matter are known to produce more ash content than the others, and will have higher mineral concentrations in the resultant AC. The levels of P, and K were much higher in the AC than other metals. This may have been due to the high ratio of pig dung to the other feedstocks (PKS, and bamboo), as similar reports of increased P, and K contents were observed in AC produced from pig dung feedstock (Lima and Marshal, 2007). It is therefore expected that the incorporation of the AC into poultry feeds will result in the increase in P, K, and Ca contents of the feeds.

4.2 Physical Characteristics, Proximate, and Mineral Composition of Layer Diets Supplemented with Activated Charcoal

In this section of the study, five (5) layer diets were formulated, and evaluated with the experimental laying birds. The diets L4, and L5 were only different from L2, and L3 by the addition of aged palm sap. Therefore, only the physical characteristics, proximate compositions, and mineral concentrations of L1 (control), L2, and L3 were analysed.

4.2.1 Physical characteristics of experimental layer diets

Table 4.4 summarizes the results of the physical characteristics of the experimental layer diets. The results showed that inclusion of up to 1.0 g of AC per 100 g of the layer diet resulted in significant changes in the physical characteristics and pH in the diets. The BD, SG, and pH values were significantly reduced ($p<0.05$) below the values recorded in the control, and the L2. The WHC on one hand, was significantly increased ($p<0.05$) indicating that the L3 diet is relatively more acidic, less bulky, and more water absorbent than the L1, and L2. Okoli *et al.* (2009) had reported that BD reduces with increasing particle size of feed which could have resulted from the increased inclusion level of the AC in the L3 diet. Bulk density also decreases with increasing moisture content of feeds (Jiang, 2016).

The ability of ACs to absorb water especially when exposed to air as shown in table 4.4 may have therefore helped to lower the BD value in L3. Bulk density as a feed quality factor, has been reported to affect the acceptability of feed and storage volume, particularly in the digestive tract of young birds (Sundu *et al*., 2005). The BD values obtained in this study (0.610 - 0.637 g/ml) are however higher than the range of 0.29 – 0.44 g/cm^3, reported for major commercial layer diets available in the Nigerian feed industry (Omede, 2010).

The higher WHC value recorded in L3 agrees with reports of a negative correlation between BD, and WHC (Sundu *et al.*, 2005; Omede, 2010). Usually, high WHC feeds will absorb more water from the gut and may form a gel beyond the gut's holding capacity, and may also trigger satiety, feed intake reductions, and poor performance (Kyriazakis and Emmans, 1995). The range of WHC values obtained in this study (1.70 – 1.74 g water/g feed) are relatively higher than the range of 0.62-1.06 g water/g feed reported for commercial poultry layer diets in Nigeria (Omede, 2010), but are however within the range reported for most conventional feed resources used in poultry diet formulation (Omede, 2010).

The SG range values of 0.618 - 0.660 recorded in this study are higher than those reported for commercial layer rations produced in Nigeria (Omede, 2010). The significantly lower SG

Table 4.4: Physical characteristics of layer diets supplemented with activated charcoal

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	SEM
Bulk density (g/ml)	0.629^{a}	0.637^{a}	0.610^{b}	0.00
Water holding capacity (%)	170.05^{b}	169.63^{b}	173.71^{a}	0.69
Specific gravity	0.647^{b}	0.660^{a}	0.618^{c}	0.01
pH	6.20^{b}	6.28^{a}	5.90^{c}	0.06

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$
L1 = Control/ 0.0 g AC/ 100 g feed, L2 = 0.5 g AC/100 g feed, L3 = 1.00 g AC/100 g feed

of the L3 indicates that this diet may exhibit a different transit property in the gastrointestinal tract, which may or may not be beneficial to the birds.

The effect of AC addition on pH was most noticeable in the L3 compared to the other diets. Since pH values are dependent on ash mineral concentrations, it is probable that the significant reduction ($p<0.05$) in potassium content of L3 (Table 4.5) may have contributed to the lower pH since K compounds are known to contribute significantly to the alkalinity of biomass-derived AC, and ash (Duruanyim, 2017; Unamba – Oparah *et al.*, 2017). The pH values of feeds recorded in this study are generally within the range reported for laying hen diets (Soltan, 2008).

4.2.2 Proximate composition of experimental layer ration

The results of the proximate analysis of the experimental layer diets are presented in table 4.5. These results highlight the nutrient potentials of the formulated diets (Okoli, Anunobi, Obua, and Enemuo, 2003; Uchegbu *et al.*, 2008). The percentage moisture content was significantly higher in AC treated diets compared to the control although they were within the accepted limits for poultry diets in the country (SON, 2018). The higher values obtained in the AC-treated diets may be attributed to the hygroscopic property of the AC that will normally result in moisture absorption by these diets over time (Verla, Horsfall, Verla, Spiff, and Ekpete, 2012). Optimal moisture values of 12 – 15 % have been recommended for poultry feeds produced in the tropics to prevent growth of fungal organisms (Okoli *et al*., 2007; Uchegbu *et al*., 2008). The MC values of 9.15 – 11.01% obtained in this experiment will therefore not encourage microbial growth in the feed.

The crude protein (CP) content, ether extract (EE), crude fibre (CF), and total ash (TA) contents decreased significantly ($p<0.05$) with increasing inclusion levels of AC in the experimental diets. The control diet (L1) had significantly higher CP value ($p<0.05$) than the treated diets.

Table 4.5: Proximate composition of experimental layer ration

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	SEM
Percentage moisture (%)	9.15^{b}	10.97^{a}	11.01^{a}	0.31
Crude protein (%)	19.27^{a}	18.10^{b}	16.65^{c}	0.38
Crude fat (%)	4.11^{a}	2.88^{b}	2.10^{c}	0.30
Crude fibre (%)	8.27^{a}	6.91^{b}	6.09^{c}	0.32
Total ash (%)	21.15^{a}	18.79^{b}	17.10^{c}	0.59
Nitrogen free extract (%)	38.07^{b}	42.36^{a}	47.15^{a}	1.31
Metabolizable energy (kcal/kg)	2650.48^{c}	2708.88^{b}	2776.20^{a}	18.46

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

L1 = Control/ 0.0 g AC/ 100 g feed, L2 = 0.5 g AC/100 g feed, L3 = 1.00 g AC/100 g feed

The dietary inclusion of AC also progressively reduced the EE, CF, and TA contents of the diets. These results may be attributed to nutrient dilution or the binding of these nutrients by the AC in the diets. Such dilution or adsorption of nutrient due to the addition of AC in poultry diets has been reported (Wang *et al.,* 2006; Grenier and Applegate, 2013). Many adsorbent materials have also been shown to decrease the concentrations of nitrogen-bearing substances in the diet without adversely affecting production performance of animals (Machacek *et al.,* 2010).

The range values for CP value (16.65 – 19.27%), EE (2.10 – 4.11%), and CF (6.09 – 8.27%) recorded in the present study were within the normal values for tropical layer rations (Bukar and Saeed, 2014; SON, 2018), while the ash content value range of 17.10 – 21.16% was higher than the reported values for most commercial layer rations sold in Nigeria (Uchegbu *et al.,* 2008; Bukar and Saeed, 2014). Ash content of the poultry feed relates to the inorganic mineral content. Higher ash content will generally mean adequate minerals capable of sustaining normal eggshell formation, and characteristics (Esonu, 2015).

The nitrogen free extract (NFE) and metabolizable energy (ME) values however increased with increasing AC addition in the layer diets. The values obtained were significantly higher ($p<0.05$) in AC-supplemented diets than in the control. These increases in the NFE, are reflective of the decreasing values of other parameters that make up the compositional analysis. Although AC may be a combustible energy source, this energy is usually not in the form readily available to the birds. The energy may however be available to the microbiota in the poultry gut. The metabolizable energy values obtained across the treatment ranges from 2650.48 – 2776.20 Kcal/kg and were also within the minimum recommended value of 2600 kcal/kg for laying birds (SON, 2018).

4.2.3 Mineral composition of experimental layer diets supplemented with activated charcoal

The mineral concentrations in the layer diets summarized in table 4.6 showed that calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), and sodium (Na) were the most abundant macro-minerals in the layer diets. Among the micro-minerals, the most abundant ones were iron (Fe) followed by manganese (Mn), zinc (Zn), chlorine (Cl), and copper (Cu). Thus, the order of mineral abundance was Ca > P> K>Mg> Na> Fe> Mn> Zn>Cl> Cu.

Overall, the supplementation of AC at 0.5 g/100 g diets resulted in either significant reduction ($p<0.05$) in the dietary values of some minerals (Mg, K, P, Mn, Fe, Cu, and Zn) or significant increase ($p<0.05$) in others (Ca, Na, and Cl) beyond the control values. This result indicates that the 0.5 g/100 g diet supplementation (L2) may represent the optimal level of AC inclusion in layer diets. The further variations from the control values observed in the 1.0 g/100 g diet supplementation level (L3) may therefore not be optimal.

Across treatments, the Ca values recorded in this study are however generally below the Standard Organization of Nigeria (SON) recommended value for layer rations (SON, 2018), while the phosphorus value is slightly lower in the L3, and slightly higher in L1, and L2 diets than the minimum P requirement (4500 mg/kg) for laying birds. Excessive calcium or phosphorus in the diet may interfere with their absorption (Maynard *et al.,* 1979), and may adversely affect egg production, and shell quality due to alterations in the acid-base balance of the birds (Moreki, 2005). All the AC supplemented diets recorded much higher Na concentration than the SON recommended values (SON, 2018), while the K, and Mg concentrations are optimal for laying hens.

Among the micro-minerals, it was observed that supplementing AC at 1% resulted in a mild lowering of Mn concentration (52.37 mg/kg) in L3 diet compared to the other groups whose

Table 4.6: Mineral composition of experimental layer ration supplemented with activated charcoal

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	SEM
Calcium (mg/kg)	12041.48 ^{c}	12454.74 ^{b}	13197.93 ^{a}	169.17
Magnesium (mg/kg)	2656.01 ^{a}	2110.03 ^{b}	1957.21 ^{c}	106.09
Potassium (mg/kg)	5877.52 ^{a}	5371.94 ^{b}	4986.42 ^{c}	129.22
Phosphorus (mg/kg)	7012.85 ^{a}	5805.26 ^{b}	3907.79 ^{c}	451.94
Sodium (mg/kg)	1830.60 ^{c}	2580.46 ^{b}	3474.50 ^{a}	241.46
Manganese (mg/kg)	90.31 ^{a}	73.57 ^{b}	52.37 ^{c}	5.49
Iron (mg/kg)	718.55 ^{a}	553.75 ^{b}	385.90 ^{c}	48.04
Copper (mg/kg)	10.35 ^{a}	9.00 ^{b}	8.36 ^{c}	0.30
Zinc (mg/kg)	60.91 ^{a}	55.71 ^{b}	37.43 ^{c}	3.56
Chlorine (mg/kg)	11.03 ^{c}	12.78 ^{b}	15.64 ^{a}	0.67

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

L1 = Control/ 0.0 g AC/ 100 g feed, L2 = 0.5 g AC/100 g feed, L3 = 1.00 g AC/100 g feed

values fell within the recommended value of 60 mg/kg (SON, 2018). The control diet also had more adequate Zn concentration than the treated diets whose values fell below the SON values for laying hen. There was however a significant decrease in Fe from 718.55 mg/kg in the control feed to 385.90 mg/kg in L3 with the values across groups being much higher than the SON standard. Similar results were also obtained for copper. These mineral values may be linked to the mineral profile of the pig dung used in the AC production (Okoli *et al.*, 2019).

4.2.4 Mineral ratios, and dietary electrolyte balance of layer ration supplemented with activated charcoal

The Ca/P, and Na/K ratios of the experimental diets as presented in table 4.7 increased significantly ($p<0.05$) with increasing inclusion level of AC in the diets compared to the control. Hence, the L3 diet had the highest values followed by L2. The L1 or control diet had significantly higher P concentration beyond the maximum requirement in diets for laying hen (Table 4.6), and without a corresponding increase in Ca concentration which influenced the Ca/P ratio. Overall, the Ca/P ratios were generally low across treatments when compared to the 13: 1, and 8:1 recommended by NRC (1994), and SON (2018) respectively for laying hen. The low Ca/P ratios obtained in the study may be because of the low calcium concentrations of the diets (Table 4.6) and may therefore influence the laying performance of the experimental birds (Esonu, 2015).

The NRC (1994) recommended requirement for layer rations is a Na/K ratio of 0.6, indicating that only the L3 diet had Na/K ratio comparable to the recommended ratio, while L1, and L2 were lower. The variations are probably due to the Na and K contents of the individual diets which were generally high compared to SON (2018) standard. The higher concentrations of Na (0.18 – 0.26%), and K (0.50 – 0.59%) like the range values reported

Table 4.7: Mineral ratios and the dietary electrolyte balance of the experimental layer diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	SEM
Ca/P ratio	1.72^{c}	2.15^{b}	3.38^{a}	0.25
Na/K ratio	0.31^{c}	0.48^{b}	0.70^{a}	0.06
dEB (Meq/kg)	228.81^{c}	249.57^{b}	278.51^{a}	7.21

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

in this study have however been found to improve the performance of broilers, especially in hot tropical regions (Borges *et al.,* 2007).

The dEB values of the experimental layer diets increased significantly ($p<0.05$) with increasing AC supplementation in the diets. This increase is obviously mediated by the significant increases in the Na concentrations of the AC supplemented diets. The values obtained are within the range (161 – 329 mEq) reported by Unamba-Oparah *et al.* (2017) for commercial layer rations produced in Nigeria, and can sustain optimal layer performance, and egg quality. Gezen, Eren, and Deniz (2005), observed that the dEB level of 256 mEq/kg caused improvement in eggshell quality, and strength, and corrected metabolic acidosis resulting from egg production. A dEB range of 230 to 250 mEq/kg was suggested to be adequate in the diets of the laying hen, regardless of heat stress or aging (Senkoyln *et al.,* 2005). Increased laying rate or hen day production percentage has also been associated with dEB status within the range of 230 – 250 mEq/kg (Kuchinski *et al.,* 1999; Chiericato, Rizzi and Zakaria, 2004). Therefore, AC-treated diets have better dEB values (249.57- 278.51) compared to the control (228.81 mEq/kg), and may translate to better laying rates, and significant improvements in eggshell quality.

4.3 Performance Characteristic of Laying Hen Fed Activated Charcoal (AC), and Activated Charcoal-Aged Palm Sap (ACAPS) Supplemented Diets

4.3.1 Laying performance of hen fed activated charcoal and ACAPS supplemented diets

Table 4.8 represents the laying performance results of the hens fed varying dietary levels of the AC, and ACAPS in their diets. The average initial liveweights of birds were similar between the treatments, and control groups at the beginning of the experiment. Generally, all the groups recorded reduced bodyweight due to egg laying during the 12 weeks of the experiment. However, birds fed treated diets recorded superior final bodyweight, and weight change values compared to the control at the end of the 12 weeks. Specifically, ACAPS group (L4 and L5) recorded the highest final weights, and lowest reduction in weight compared to AC group (L2 and L3), and the control. The ratio of energy intake to energy expenditure determines the body weight. An imbalance between energy intake, and expenditure usually results in a change in body weight (Esonu, 2015). The ACAPS dietary supplementation significantly reduced ($p<0.05$) the loss in weight because of egg laying intensity. A similar but non-significant effect was also reported by Kutlu *et al.* (2001), when charcoal was fed at incremental levels to laying hens. Combination of AC, dietary organic acids, and probiotics supplementation has been reported to improve/maintain body weight in laying hen (Yamauchi *et al.*, 2010; Rattanawut *et al.*, 2017), and may explain the current effects of ACAPS since it also contains AC, yeast and lactobacilli probiotics, some alcohol, and organic acids.

The treated birds also recorded superior feed intake compared to the control. The increased feed intake with increasing dietary supplementation of AC, and ACAPS must have resulted from improved nutrient digestibility which invariably helped to maintain the body weights of the treated birds (Samanya, and Yamauchi, 2002). Higher levels of AC, and or organic acid supplementations have been reported to aid the intake, and digestion of diets whose fibre fractions may be ordinarily difficult to digest (Kutlu *et al.*, 2001). Kalus *et al.* (2020) reported increased intake in laying hens fed varying levels of beech wood biochar in their diets. The addition of 0.4% bamboo vinegar in the diets of laying ducks has also been reported to improve feed consumption, and feed conversion efficiency (Rattanawut *et al.*, 2019). The AC, and ACAPS treated birds recorded superior hen-day production percentages compared to the control. Figure IV highlights the potentials of AC and ACAPS supplemented diets to enhance egg laying frequency. The combination of 1% AC, and aged palm sap vinegar (L5) increased laying rate, producing 12.84% more eggs than the control, while the rest of the treatment groups produced 6.57% (L2), 8.28% (L3), and 8.68% (L4) more eggs than the control (Fig. IV). This agrees with earlier reports of improved FCR, and egg production

Table 4.8: Layer performance of hens fed activated charcoal and ACAPS

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Initial weight (g)	1714.33	1684.00	1724.00	1677.33	1724.67	20.53
Final weight (g)	1567.67 ^{b}	1623.33 ^{ab}	1660.00 ^{a}	1639.33 ^{ab}	1701.67 ^{a}	15.09
Weight change (g)	-146.00 ^{b}	-60.67 ^{ab}	-64.00 ^{ab}	-31.33 ^{a}	-18.00 ^{a}	52.70
Average daily feed intake (g)	112.86 ^{b}	118.69 ^{ab}	118.50 ^{ab}	118.07 ^{ab}	120.86 ^{a}	0.98
Hen-day production (%)	78.72 ^{b}	83.89 ^{ab}	85.24 ^{ab}	85.55 ^{ab}	88.83 ^{a}	1.32
Egg mass	45.19	49.07	50.22	50.96	50.40	0.99
FCR	2.52	2.42	2.36	2.32	2.40	0.05

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Legend

FCR = feed conversion ratio, L1 0.0 g AC/100 g feed,

L2 = 0.5 g AC/100 g feed, L3 = 1.0 g AC/100 g feed,

L4 = 0.5 g ACAPS/100 g feed, L5 = 1.0 g ACAPS/100 g feed.

in laying hens fed dietary bamboo charcoal, and or its vinegar solution (Yamauchi *et al.,* 2010; Gerlach and Schimdt, 2012). Similar trends were also observed by Kalus *et al.* (2020) who reported improved laying performance with dietary beechwood charcoal supplementation in laying hen.

Reports on the impacts of AC supplementation on percentage hen-day production however differ across study results. Prasai *et al.* (2016) observed no impact of AC feeding on laying rate, but in a later study however, reported improved FCR, egg production, egg weight, and egg mass with biochar amendment in the diets of laying hen (Prasai *et al.,* 2017). Rattanawut *et al.* (2017) also observed that the feeding of laying hen with a blend of bamboo charcoal, and vinegar had no significant impact on egg production. The addition of AC in layer diets neither affected nor significantly improved laying performance of laying hen except for decreased frequency of eggshell cracking (Kutlu *et al.*, 2001). It is therefore possible that the inherent characteristics of the AC, and organic acid used in such experiments are of critical importance to the final outcome. Again, the addition of probiotic effects of the yeast, and the numerous LABs present in the ACAPS would have contributed to the superior results recorded in this study (Agboola *et al.*, 2015; Youssef *et al.*, 2017).

No statistical effect was observed for the FCR and egg mass values during the entire experimental period. However, the values were numerically improved in all the AC treated groups compared to the control group. Gerlach and Schmidt (2012), and Kalus *et al.* (2020) reported similar results in laying hen fed bamboo charcoal/vinegar, and beechwood charcoal respectively. Prasai *et al.* (2017) also recorded non-significant improvements in laying rate, and egg mass, but superior FCR in laying hen fed diets treated with absorbents (biochar, zeolite, and bentonite) relative to the control.

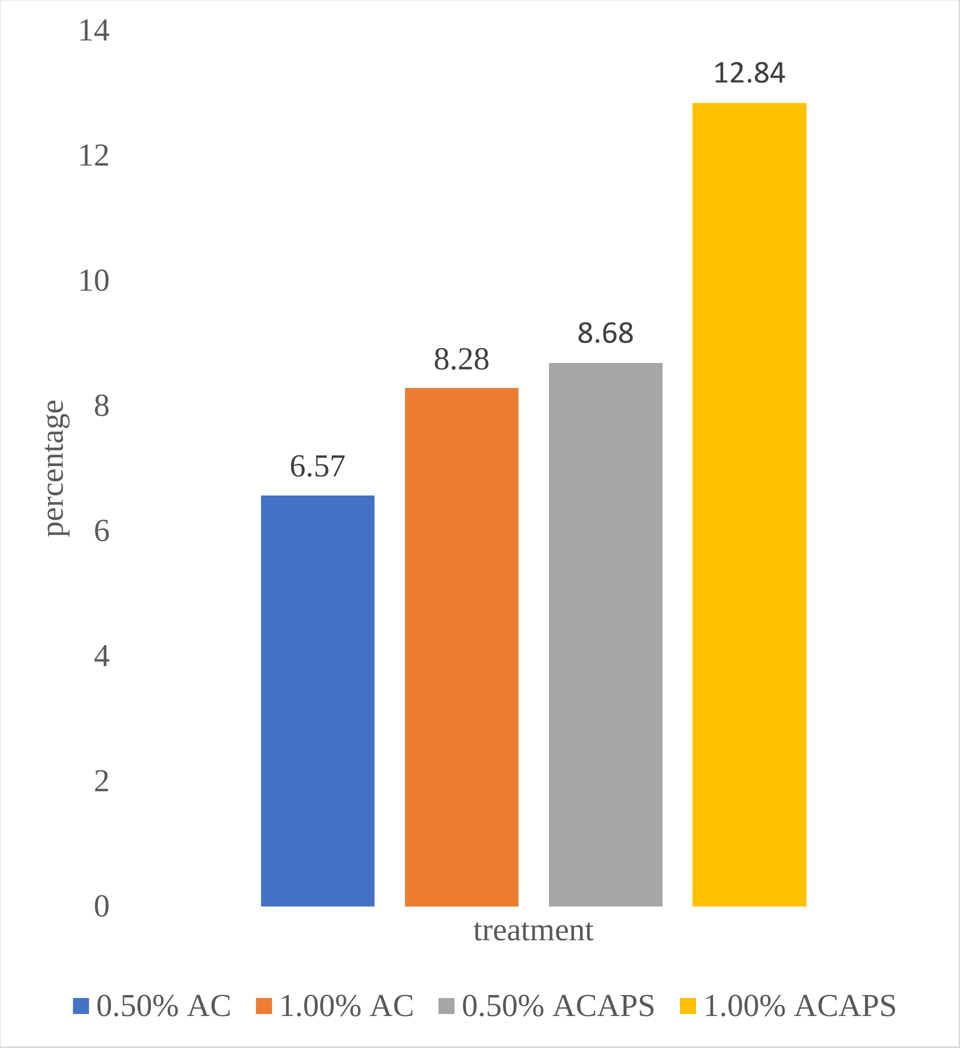

Figure IV: Percentage increment in hen-day production as a result of AC and ACAPS supplementation.

4.3.2 External quality characteristics of egg laid by hens fed AC and ACAPS supplemented diets

All the external egg characteristics results obtained in this experiment (Table 4.9) were not significantly affected by the treatments (p>0.05), indicating that the AC supplements have no negative effect on egg shape, and shell quality parameters. This is in line with several other studies that reported no effects with supplemental AC or vinegar feeding (Kutlu *et al.,* 2001; Rattanawut *et al.,* 2019). However, some egg characteristics such as the average egg weight, egg shape index, and eggshell thickness had some numerical but non-significant improvements, especially in eggs laid by the hen fed the AC treated diets. This is probably because of better dietary mineral utilization by these birds (Table 4.6). According to Taylor and Dacke (1984), eggshell thickness increases distinctly as the daily intake of calcium from the diets increases. Improved eggshell strength, and reduced frequency of cracked eggs have been reported with AC, and vinegar feeding in laying birds, although with no significant improvements in egg production or other egg quality parameters (Ayanwale *et al.,* 2006, Kim *et al.,* 2006; Rattanawut *et al.,* 2017).

4.3.3 Internal quality characteristics of the eggs laid by hens fed AC and ACAPS supplemented diets

Data representing internal quality characteristics of the eggs laid by the hen fed AC, and ACAPS supplemented diets are presented in Table 4.10. Generally, AC supplementation in the diets had no significant effects on all the albumen characteristics (p>0.05) but had significant effects on most of the yolk characteristics, indicating that the effects were mostly on the yolk. All the eggs however recorded statistically similar yolk length, and percentage values, while L2 recorded significantly different yolk height, weight, index, and colour values from the other groups. Compared to the control, birds on AC treated diets recorded

Table 4.9: External characteristics of eggs laid by hen fed dietary AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Average egg weight (g)	57.25	58.50	58.87	59.57	56.70	0.48
Egg length (mm)	56.12	55.96	55.84	55.91	55.38	0.18
Egg width (mm)	42.05	42.66	42.84	43.03	42.03	0.16
Shape index	74.93	76.23	76.73	76.97	75.91	0.35
Shell weight (g)	7.41	7.50	7.50	7.83	7.23	0.09
Shell percentage (%)	12.97	12.82	12.74	13.15	12.75	0.14
Shell thickness (mm)	0.33	0.35	0.35	0.35	0.35	0.01

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Table 4.10: Internal quality characteristics of eggs laid by hens fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Albumen height (mm)	7.76	8.10	7.71	8.35	7.73	0.12
Yolk height (mm)	16.61 ^{a}	15.57 ^{b}	16.74 ^{a}	17.09 ^{a}	16.84 ^{a}	0.16
Albumen weight (g)	34.30	34.43	34.77	34.87	33.27	0.40
Yolk weight (g)	15.17 ^{b}	16.13 ^{a}	16.27 ^{a}	15.83 ^{ab}	15.63 ^{ab}	0.14
Albumen length (mm)	80.59	80.83	81.38	82.25	82.23	0.69
Yolk length (mm)	41.31	41.55	42.25	41.54	41.33	0.17
Albumen width (mm)	64.33	62.99	64.20	64.45	65.17	0.47
Yolk width (mm)	40.01 ^{b}	40.44 ^{ab}	41.05 ^{a}	40.53 ^{ab}	40.09 ^{ab}	0.15
Percentage albumen (%)	59.84	58.85	59.04	58.53	58.66	0.29
Percentage yolk (%)	26.51	27.57	27.65	26.59	27.58	0.20
Albumen index	10.74	11.28	10.59	11.39	10.49	0.20
Yolk index	41.53 ^{a}	38.51 ^{b}	40.78 ^{a}	42.16 ^{a}	42.01 ^{a}	0.41
Yolk colour	6.88 ^{ab}	6.40 ^{c}	7.07 ^{ab}	6.70 ^{bc}	7.23 ^{a}	0.09
Haugh unit	88.37	89.91	87.63	91.03	88.42	0.65

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

better yolk weight values. The L2 and L3 birds laid eggs with significantly higher ($p<0.05$) yolk weight but did not differ ($p>0.05$) from those on ACAPS supplemented (L4 and L5) diets. Yolk weights of ACAPS supplemented diets did not also differ from those of the control. The production implication is that all the diets supported sufficient nutrient reserves in the yolk, capable of sustaining normal egg formation (Odunsi, Ogunleke, Alagbe, and Ajani, 2002). The yolk width values of L3 eggs were also higher than the control values, and also similar to the values recorded in the other AC treated groups.

Laying hens on diets L5 had eggs with the deepest yolk colour value similar to those laid by the birds on the L3, and control diets but significantly different from L2 and L4 egg values. The L4 birds also laid eggs with better yolk colour scores than the L2 eggs which were the lightest. The yolk colour trend is very interesting and does not agree with existing reports of decreased yolk colour scores with increased supplementation of feed adsorbents such as activated charcoal in laying hen (Prasai *et al.*, 2017). Increased colour intensity of egg yolk has been reportedly influenced by higher dEB status of the diet as shown in table 4.7 for L3 diet (Chiericato *et al.*, 2004). Deep coloration of egg yolk is influenced by the presence of bioactive compounds such as carotenoids, xanthophyll etc., in the diets, and has been reported to influence consumer preference (Arimboor *et al.*, 2015). Therefore, the feeding of L3, and L5 diets will help to produce eggs with better customer appeal compared to the other groups. However, the colour range of 6.40 – 7.23 obtained in this study conforms with the range of 6.08 to 9.04 reported for different absorbents in layer diets (Prasai *et al.*, 2017), and surpassed the minimum acceptable yolk colour score value of 4.0 (Hy-Line 2016).

4.4 Economics of Producing Eggs from Hens fed AC and ACAPS Supplemented Diets.

Data representing the economics of producing eggs by the hens fed diets supplemented with AC, and ACAPS are presented in Table 4.11. Treatment effects resulting from AC supplementation in the diets of laying hen were pronounced in the total feed consumed per bird, cost of feed consumed, and total egg produced per bird. The parameters all increased progressively across groups with L5 particularly recording significantly higher values ($p<0.05$) than the control. Overall, the cost of feeding increased with increased feed intake which was also compensated with increased egg production by these groups. This is reflective of the improvements in the hen-day production highlighted in figure IV.

The feed cost per kilogramme egg produced by the laying hen in this study did not differ significantly between the AC, ACAPS, and the control birds. However, numeric decrease in feed cost per kilogramme egg was witnessed in treated birds owing to their better FCR compared to the control. This also translated to increased revenue, and profits margin from the supplementations of AC, and ACAPS in the laying hen diets, although the differences between these, and the control group values were not significant.

Figure V shows the percentage differences in the mean economic indices of the hen. Although the AC, and ACAPS treated hen had percentage increases (4.53 – 6.99%) in their total feed intake consumed per hen than the control, the percentage differences in the feed cost per kilogramme of egg produced by these hens were lower than the control value with 1.00% AC (L3) and 0.50% ACAPS (L4) recording the lowest percentage difference in feed cost per kg of egg (-6.47 and -8.10% respectively). The graph also highlights the percentage increment in profit resulting from the AC (7.32 – 9.93%), and ACAPS (10.50 – 15.84%) supplementation in these groups compared to the control.

Table 4.11: Cost-benefit analysis of laying hen fed varying dietary levels of ACAPS

Parameter	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Feed cost/ KG (N)^{a*}	112.15	112.15	112.15	112.15	112.15	0.00
Total feed intake/bird (kg)^{b**}	10.16 ^{b}	10.67 ^{ab}	10.68 ^{ab}	10.62 ^{ab}	10.87 ^{a}	0.09
Cost of feed consumed (N)^{c***}	1139.07 ^{b}	1197.02^{ab}	1195.89 ^{ab}	1191.41^{ab}	1219.82 ^{a}	9.90
Total egg produced/bird θβ	66.13 ^{b}	70.47 ^{ab}	71.60 ^{ab}	71.73 ^{ab}	74.61 ^{a}	1.11
Feed cost per kg egg produced (N)	282.99	271.69	264.67	260.08	270.64	5.10
Revenue (N) λ	3306.67	3523.33	3580.00	3586.67	3730.67	55.27
Profit	2167.60	2326.32	2384.11	2395.26	2510.85	49.98

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Feed cost /kg = a*; Total feed intake =**; Cost of feed consumed (^{c***}) = a* x **; (θβ) = Total egg produced per hen; Revenue (λ) = θβ x retail cost of an egg @ N50 naira; Profit = λ-c***. feed cost /dozen egg = FCR x a*

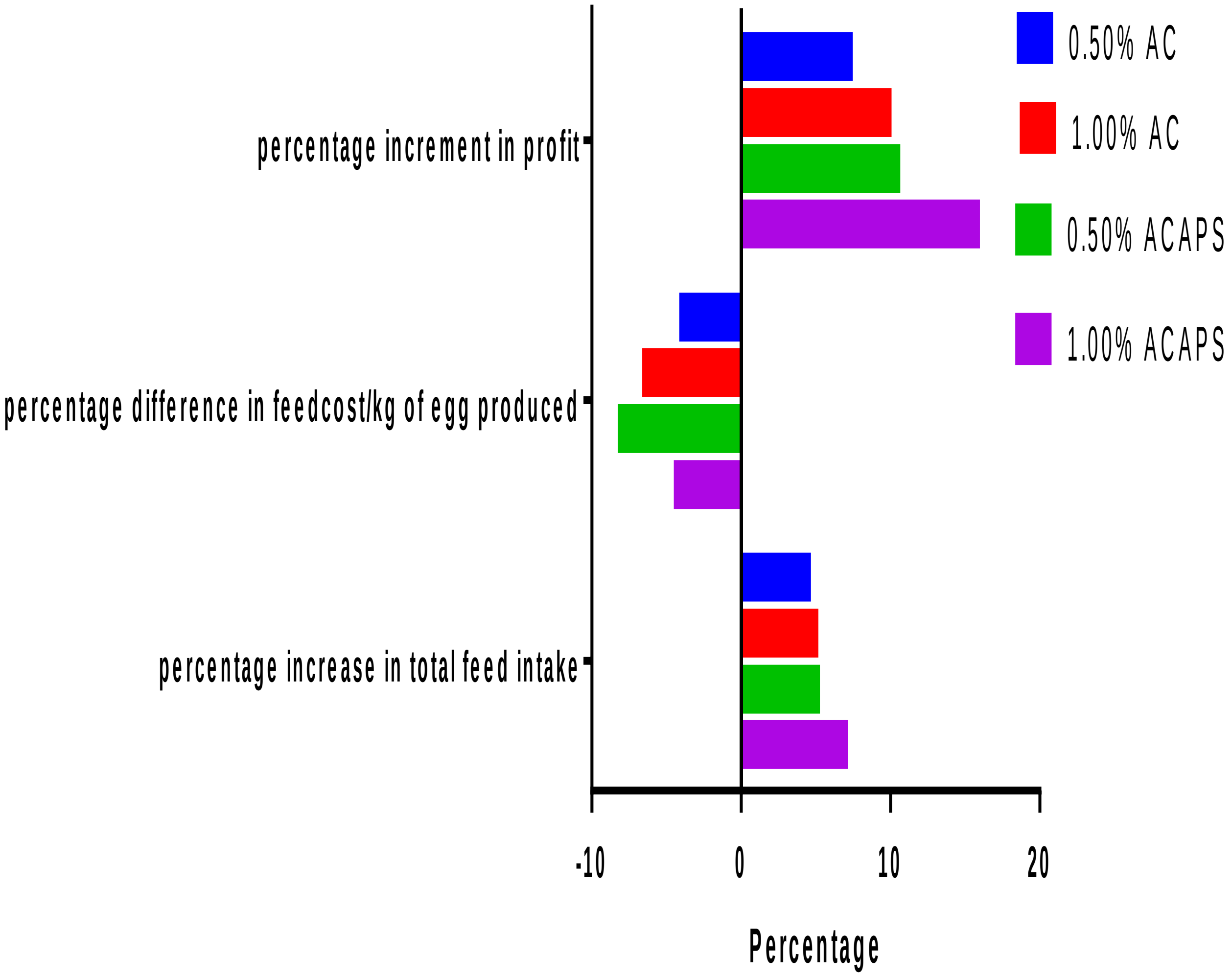

Fig. V. Percentage differences from the control of economic indices of eggs produced by AC, and ACAPS treated birds

4.5 Haematological Characteristics of Laying Hen fed AC and ACAPS Supplemented Diets

Haematological, and blood biochemistry analysis are valuable tools deployed in the assessment of metabolic, and nutritional disorders, disease diagnosis, and clinical monitoring in the avian species (Sharma, Kumar, Singh, and Vasishta, 2015). A good interpretation of these parameters however requires appropriate reference values of each species to eliminate the effect of species differences (Lanzarot, Barahona, Andrés, Fernández-García, and Rodríguez, 2005).

The parameters routinely assessed include, the red blood cells (RBC), haemoglobin (Hb), pack cell volume (PCV), platelets count, blood coagulation time (BCT), white blood cell (WBC), and WBC differentials. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are also calculated from the RBC, HB, and PCV values.

4.5.1 Effect of AC and ACAPS supplementation on the blood parameters of laying hens

The results of the Hb, PCV, RBC, MCV, MCH, MCHC and BCT are presented in table 4.12. Generally, the inclusion of AC and ACAPS in the diets of the laying hen had significant effects on the haematological parameters measured. The dietary supplementation of AC has been reported to improve haematological parameters in poultry. In contrast, dietary AC supplementation resulted in significantly lower ($p<0.05$) blood values in the layer hen. Haematological values decreased with increasing inclusion levels of the AC, and ACAPS in the diets. For example, at 1.0% inclusion (L3) of AC, the Hb, PCV, and RBC values became significantly lower ($p<0.05$) than the control, and the L2 values. Similarly, the L4 and L5 (0.5, and 1.0% ACAPS respectively) values were significantly lower than the control, L2, and L3 values. According to Muhammad and Oloyode (2009), decreases in haemoglobin

Table 4.12: Haematological indices of layer birds fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Hb (g/dl)	12.70 ^{a}	12.50 ^{a}	12.17 ^{b}	11.10 ^{c}	11.10 ^{c}	0.19
PCV (%)	38.07 ^{a}	38.00 ^{a}	36.00 ^{b}	33.33 ^{c}	33.40 ^{c}	0.58
RBC (X10^{6} µl)	6.30 ^{a}	6.30 ^{a}	5.70 ^{b}	4.70 ^{c}	4.50 ^{c}	0.21
MCV (fl)	123.68 ^{a}	121.60 ^{b}	119.79 ^{b}	112.85 ^{c}	108.80 ^{d}	1.52
MCH (ρg)	24.16 ^{a}	24.00 ^{a}	23.47 ^{b}	22.40 ^{c}	20.16 ^{d}	0.40
MCHC (ρg)	28.60 ^{c}	27.70 ^{d}	26.90 ^{e}	29.83 ^{a}	29.00 ^{b}	0.27
BCT (sec)	37.00 ^{a}	35.00 ^{b}	32.00 ^{c}	27.00 ^{d}	25.00 ^{e}	1.23

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Hb = haemoglobin, PCV = pack cell volume, RBC = red blood cells count, WBC = white blood cell, MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, BCT = blood coagulation time concentrations could be accompanied by depletion in the red blood cell count, and packed cell volume which agrees with our decreased levels of these indices. Increased charcoal addition in the layer diets significantly reduced important micro mineral concentrations, especially iron, and copper in the experimental diets (Table 4.6) which may have affected blood formation possibly because of reduced mineral availability from the diets. Higher levels, and long-term administration of adsorbents such as AC may affect the uptake of bio-active substances such as vitamins, minerals, and other specific-action substances from the diets, and thus influence nutrient metabolism indicators, and haematopoiesis (Machacek *et al.,* 2010). It is however interesting to note that the addition of aged – palm wine to the AC did not ameliorate this effect but rather seemed to potentiate it.

The adequate supply of dietary protein, minerals (iron, and copper), and vitamins are principal to the sustenance of normal red cell production (Nyaulingo, 2013). According to Mackenzie *et al.* (2008), dietary iron is critical for oxygen transport, energy production, and DNA synthesis. Therefore, haematological efficiency is assured by adequate supply of these metals in the diet. Nevertheless, the Hb, PCV, and RBC values obtained in this study are similar to the reference values reported for chicken (Bounos and Steadman, 2000; Harr, Harrison, and Lightfoot, 2005; Wikivet, 2013; Al – Nedawi, 2018).

The erythrocytic indices such as MCV, MCH, and MCHC usually aid the characterization of anaemic states in animals (Aiello and Mays, 1998). According to Ochei and Kolhatkav (2007), a decrease in their values indicates iron deficiency, and macrocytic anaemia. The MCV, and MCH values also decreased progressively with increased AC, and ACAPS inclusion, whereas the MCHC value improved with the ACAPS inclusion (L4 and L5). The L3 values for the MCV, and MCH were thus significantly higher ($p<0.05$) than the L4 and L5 values. Again, all the values were within the limits reported for normal chicken (Bounos and Steadman, 2000; Al-Nedawi *et al.,* 2018), which is suggestive of normal amounts, and concentrations of haemoglobin in the red cells, and indicative of normocytic, and normochromic cells (Okeudo, Okoli, and Igwe, 2003). The key minerals responsible for blood formation, and maintenance of its physiological integrity are Fe, Cu, and Co. It would seem therefore that the haematological results obtained in this study are influenced mostly by the Fe, and Cu concentrations of the diets.

Blood coagulation time (BCT) of the experimental birds decreased progressively with increasing levels of AC, and ACAPS inclusion in the diets. BCT was highest in the control birds but lowest for L4 and L5 birds. This result suggests that AC contains minerals such as calcium in the forms that reduce BCT, which also increased in AC treated diets (Table 4.6) and has been reported to enhance blood clotting ability (Waldroup, 1997). It also means that the treated diets enhanced thromboplastic substances that caused rapid coagulation of blood upon withdrawal. Coagulation time of blood is increased when vitamin K is deficient in veinous blood, as the vitamin is vital to the synthesis of prothrombin. Similar reductions in BCT were also reported by Ohanaka, (2016) in broilers fed diets containing palm kernel shell ash in their ration. The BCT range of 28 – 37 sec recorded in this study are however within the range reported by Doerr and Hamilton (1981), but much lower than the BCT range of 161 – 223 sec reported for laying hens fed bambara seed offal as a replacement for soya bean (Okonkwo and Esiegwu, 2017).

4.5.2 WBC and differential counts in laying birds fed AC and ACAPS supplemented diets

Table 4.13 shows that AC supplementation had no effect on the white blood cell (WBC) counts, and its differential values. ACAPS supplementation however resulted in significant ($p<0.05$) reductions in WBC counts below the control, and AC supplementation values. There were no treatment effects on lymphocyte values due to the AC and ACAPS

Table 4.13: WBC differentials of laying hen fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
WBC (X10^3 µl)	5.20^a	5.20^a	5.10^a	4.10^b	4.00^b	0.15
Lymphocyte (%)	28.00	27.00	27.00	27.00	26.33	0.30
Heterophils (%)	65.67^a	64.00^{ab}	64.00^{ab}	62.00^b	62.00^b	0.46
Eosinophil (%)	0.00^b	1.33^b	3.00^a	3.33^a	3.00^a	0.39
Monocyte (%)	6.33^{ab}	7.67^{ab}	6.00^b	7.67^{ab}	8.67^a	0.37
Basophil (%)		-	-	-	-	-

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

supplementations. The normal WBC counts indicate optimal immune system, and phagocytic functions in the hens (Robert *et al*., 2003). The values obtained in this study are however within the normal range for chicken (Mitruka and Rawnsley, 1997). The supplementations of AC also did reduce the heterophil counts in the hen. High levels of circulating heterophils in the blood is indicative of bacterial infection (Guyton and Hall, 2006). Eosinophil count is vital in the defence of the body against parasitic infections, while monocytes perform important defence function like heterophils but remain longer in circulation. Increased number of monocytes is usually a response to infections, while a decrease in the number of monocyte count is not usually identified with any specific disease processes. All the differential counts obtained in this study were also within normal range, while the absence of basophil is an indication that none of the birds in the groups was exposed to stress factors (Ekunseitan *et al.*, 2017; Maxwell, Hocking, and Robertson, 1992).

4.6 Serum Biochemical Indices of Laying Hen Fed AC and ACAPS Supplemented Diets

4.6.1 Compositions of serum nutrients and metabolites in laying hen fed AC and ACAPS supplemented diets

Table 4.14 shows the results of serum nutrients and metabolites measurements. These results revealed that AC, and ACAPS inclusion in the diets influenced the serum nutrients values which were significantly different across most treatments. Total serum protein (TSP) values were significantly higher ($p<0.05$), and similar in the control, and the AC treated diets, than in the ACAPS treated birds. The albumin values also followed similar trends although significant effects ($p<0.05$) were observed from the 1.0% AC supplementation (L3), and in all the ACAPS groups. The globulin concentration followed the opposite trend, and increased progressively in the AC, and ACAPS treated birds. The values recorded in the treated birds were significantly higher than the control values ($p<0.05$).

Table 4.14: Serum chemistry of laying hen fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Total serum protein (g/dl)	7.50^{a}	7.50^{a}	7.40^{a}	7.00^{b}	6.72^{c}	0.88
Albumin (g/dl)	4.20^{a}	4.10^{a}	3.87^{b}	3.52^{c}	3.23^{d}	0.98
Globulin (g/dl)	3.30^{b}	3.40^{ab}	3.50^{a}	3.53^{a}	3.49^{a}	0.30
Albumin/Globulin ratio	1.27^{a}	1.21^{b}	1.10^{c}	1.00^{d}	0.92^{e}	0.03
Urea (mg/dl)	20.50^{e}	20.90^{d}	21.30^{c}	22.00^{b}	24.20^{a}	0.35
Glucose (mg/dl)	144.00^{a}	142.00^{ab}	140.00^{b}	136.00^{c}	130.00^{d}	1.39
Cholesterol (mg/dl)	113.00^{a}	109.00^{b}	106.00^{c}	104.00^{c}	96.33^{d}	1.53
Creatinine (pg/dl)	1.10^{c}	1.20^{bc}	1.20^{bc}	1.30^{ab}	1.40^{a}	0.03

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Serum proteins in avian species are important indicators of their health status. In female birds, a significant increase in total serum protein concentration occurs just prior to egg laying and has been attributed to an oestrogen-induced increase in globulins (Khawaja, Khan, Mukhtar, Parveen, and Fareed, 2013). Total protein, and albumin values indicate availability of proteins, and their serum concentrations will usually decline in the face of dietary deficiency. Since blood proteins are synthesized by the liver hepatocytes (Saba, Oridupa, and Ofuegbe, 2009), low serum concentrations are again, indicative of liver degeneration (Thapa and Walia, 2007), usually induced by the continuous synthesis of vitellogenin in the liver of the egg laying birds (Saraswati, Manalu, Ekastuti, and Kusumorini, 2013). Therefore, the lower values obtained in the ACAPS treated birds may be reflecting the physiological impacts of increased laying intensity which were observed in these birds. More importantly, the AC supplementation resulted in significant reductions in the crude protein values of the diets (Table 4.5), a phenomenon also reported with other adsorbent material treatments (Wang *et al.*, 2006; Grenier and Applegate, 2013). It is therefore possible that a significant proportion of the dietary proteins was channelled elsewhere or bound to the adsorbents, without reaching the blood stream. Wang *et al.* (2006) also observed similar decrease in blood proteins in broilers whose diets were supplemented with sorbent materials such as AC, and hydrated sodium alumino – silicate (HSCAS). Although our laying performance results (Table 4.8), and other published studies have shown no adverse effects associated with reduction of this nutrient values (Machacek *et al.*, 2010), the mechanism behind it is not clear, and therefore needs to be investigated further.

The TSP values of 6.72 – 7.5 g/dl recorded in this study are however similar to the range of 7.25 – 10.15 g/dl reported by Nwogu (2013) in hen fed plantain ash and also within the reference range for chicken (Aiello and Mays, 1998), indicating their sufficiency in sustaining or supporting optimal production performance. The significant increase in globulin values of the AC treated hen relative to the control, signifies improved immunoglobulin production. The albumin/globulin ratios (A/G) also decreased progressively across treatments such that each treatment value was significantly lower ($p<0.05$) than the next value. Thus, the AC and ACAPS values were significantly lower than the control value. Lombardi *et al.* (2020) and Bovera *et al.* (2015) reported that high globulin levels, and low albumin/globulin ratios are indicative of improvements in immune response, and disease fighting ability in the birds. The A/G ratio (0.92 – 1.27) obtained in the present study were comparable with the range reported earlier for laying hen (Zeweil, Genedy, and Bassiouni, 2006), but higher than values reported in more recent studies on laying hen (Bovera *et al.*, 2018; Lombardi *et al.*, 2020).

Blood urea, and creatinine levels increased progressively with increasing supplementation levels of AC, and ACAPS in the diets, with L5 recording the highest values, while the control recorded the least value. Serum creatinine, urea, uric acid, and electrolytes values are parameters employed in the assessment of nervous functions (Musso, Gregori, Jauregui, and Núñez, 2012). Creatinine is a metabolite derived from creatine, which is predominantly found in skeletal muscles, while urea is generated from protein catabolism, and often dependent on daily protein intake, and endogenous protein metabolism. The decreasing TSP earlier observed in this study in the ACAPS treated birds may therefore partially be as a result of increased protein catabolism into urea in these birds. The probiotic, and other micro-organism in the aged-palm wine may be partially responsible for this. Serum urea, TSP, and creatinine have been associated with the quality, and quantity of proteins in a diet (Iyayi and Tewe, 1998). The increased levels of creatinine values in the AC, and ACAPS treated birds was dose-dependent, and may therefore be linked also to the increased egg production witnessed in these groups. According to Kececi and Col (2011), serum creatinine level increases as egg production, flight expressions and other behavioural patterns intensify.

The serum glucose concentration differed across AC, and ACAPS supplementation levels, and was significantly lowered ($p<0.05$) by the ACAPS supplementation. Glucose is an essential nutrient, and the main energy source for many cells. The liver is critical in regulating the uptake, and storage of glucose through glycogenesis, glycogenolysis, and gluconeogenesis (Sharabi, Tavares, Rines, and Puigserver, 2015). The changes in glucose concentration have been linked to increases in egg laying intensity, which requires higher energy utilization (Ekunseitan *et al.,* 2017). In general, the blood glucose concentrations obtained across treatments were within the reference limits reported by Mitruka and Rawnsley (1997), and Thrall, Weiser, Allison, and Campbell (2012)

Blood cholesterol values obtained also showed significant decrease with corresponding increase in AC, and ACAPS dietary supplementation. Thus, birds on the test diets, and particularly ACAPS supplemented group, had significantly lower ($p<0.05$) serum cholesterol values relative to the control. Laying hens have been suggested to eliminate considerable serum cholesterol in the eggs (Lombardi *et al.*, 2020). The observed decrease in the serum cholesterol values may therefore be linked to the increased intensity of egg production because of AC, and ACAPS supplementation recorded in this study. Inclusion of activated charcoal in poultry diets is reported to reduce absorption capacity for dietary fat and increase its excretion in the faeces (Ayanwale *et al.,* 2006; Jiya *et al.,* 2014). Similar findings of decrease in serum cholesterol levels were also reported in laying hens fed plant ash in their diets (Nwogu, 2013). However, the range of values recorded in this study is generally within the reported normal reference range (100 – 150 mg/dl) for the chicken (Sturkie *et al.*, 2000).

4.6.2 Composition of serum enzymes in laying hen fed AC and ACAPS supplemented diets

The effects of dietary AC, and ACAPS supplementation on serum enzymes activities in the laying hens are shown in Table 4.15. Generally, the liver enzymes evaluated in this study were significantly influenced by the AC, and ACAPS supplementations. The alkaline phosphatase (ALP) concentrations increased significantly ($p<0.05$) with the increasing inclusion levels of the additives such that L5 recorded the highest level. In contrast, the alanine amino transferase (ALT), and the aspartate amino transferase (AST) concentrations were highest in the control and decreased significantly ($p<0.05$) in the L3 (1.0% AC) and all the ACAPS supplemented groups.

The liver synthesizes enzymes, proteins, and hormones, which exhibits critical influence on Ca metabolism, bone development, and egg production (Jiang, Cheng, Cui, Zhou, and Hou, 2013; Lombardi *et al.*, 2020). Liver enzymes such as ALT, ALP, and AST are important hepatic toxicity markers (Hussein, Soliman, Ali, Tawfeik, and Abdelrahim, 2013), used in the evaluation of the proper hepatic function (Ambrosy, Dunn, and Heidenreich, 2015). These enzymes are found mostly in liver, but usually leak into the blood only when the liver cells are damaged or destroyed. The presence in the blood of significant quantities of these liver enzymes therefore indicates increased liver tissue damage or injury (Saeed, Deng, and Dai, 2008; Berredjem, Reggami, Benlaifa, Berredjem, and Bouzerna, 2015).

The ALP enzyme is associated with intestinal lipid transport, and bone calcification. Increases in ALP activity is either due to physiological causes, intestinal lesions, liver dysfunctions and skeletal disorders which requires bone remodelling (Atia, Waibel, Hermes, Carlson, and Walser, 2000). It is therefore possible that the increase in ALP activity in the AC, and ACAPS treated birds is because of improved bone remodelling, and or eggshell formation. According to Huff *et al.* (1998), increased activity of ALP reflects increased availability of phosphorus.

Table 4.15: Serum enzymes activities in layers fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
ALP (iu/L)	71.00^{d}	73.00^{c}	74.00^{bc}	75.00^{ab}	76.00^{a}	0.51
ALT (iu/L)	9.30^{a}	9.10^{a}	8.60^{b}	8.00^{c}	7.80^{c}	0.16
AST (iu/L)	7.80^{a}	7.60^{a}	7.20^{b}	7.10^{b}	7.10^{b}	0.09

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

ALP = alkaline phosphatase, ALT = alanine amino transferase

AST = aspartate amino transferase

The ALP activity is also a suitable indicator of serum calcium status in animals (Reichman and Connor, 1977), and may rise due to increased calcification that accompanies fast growth in broilers or eggshell formation in laying hen (North and Bell, 1990; Harr, 2002). Therefore, the increased ALP levels especially in L4 and L5 birds signify better Ca, and P status. The ALP values obtained in this study are within normal range for chicken (CDD, 1990).

Elevated AST activities are associated with liver or muscle damage, but no particular significance is linked with low AST activity in birds (Viveros *et al*., 2002). The higher AST value observed in the hen on the control diet signifies the suboptimal utilization of feed ingredients towards growth or egg production, and therefore higher susceptibility to hepato-toxicity.

The ALT has greater diagnostic sensitivity, and more specific to liver disease than AST (Kew, 2000). Higher ALT activities beyond the upper reference limit have been associated with acute viral hepatitis, increased liver perfusion and death due to ingestion of toxins. Therefore, the higher ALT values observed in the control birds indicate relatively lower hepatic integrity. However, the AST and ALT values obtained in the study are within the ranges reported for healthy chicken.

4.6.3 Serum mineral concentrations in laying hens fed AC and ACAPS supplemented diets

The electrolytes concentrations in the sera of laying hens fed diets containing AC, and ACAPS are shown in table 4.16. Generally, the electrolyte levels, especially Ca, P, Na, K, and chloride levels decreased progressively with increases in the dietary inclusions of the AC, and ACAPS, while the reverse trend was observed for bicarbonate values. Specifically, L2, and L3 groups maintained similar ($p>0.05$) serum Ca values with the control, which were higher, and significantly different ($p<0.05$) from those of the ACAPS fed hens (L4 and L5). Serum phosphorus concentration was superior in the birds on the control diet compared to the treated birds. Similar trends were also observed for serum Na, K, and Cl, while HCO_3^- increased as a result of increased inclusion levels of AC, and ACAPS in the diets.

Dietary minerals are critical to the development of immune status, improved egg quality, and laying rate in poultry (Tronina, Korczyński, Opaliński, Dobrzański, and Kaźmierska, 2006), and may be required in large amounts during production. Therefore, the concentrations of blood serum minerals are valuable indicators of mineral status in poultry, especially during intense skeletal development (young birds) or egg formation (Dobrzanski, Opaliński, Hoffmann, Bubel, and Pogoda-Sewerniak, 2011). Serum Ca, and P levels are critical during egg formation, and maintenance of skeletal integrity. Their concentration in the blood serum is largely dependent on feed supply, chemical form of the minerals, and uniformity in mixing in rations (Kurtoglu, Kurtoglu, and Balevi, 2007; Dobrzański *et al.*, 2011). Pelicia *et al.* (2009) observed increased serum Ca concentration in laying hens fed increased dietary Ca. In contrast, Lombardi *et al.* (2020), observed increased Ca mineral absorption in low dietary concentrations, while higher Ca levels in the diet, reduced its absorption.

From our feed composition, and feed mineral data (Tables 4.5 and 4.6), the AC supplemented diets had higher energy, and calcium levels more than the control. Therefore, the reduced serum Ca concentration could not be as a result of dietary deficiency, but due to the physiological processes involved in eggshell formation, and egg laying. Studies have shown significant decreases in serum mineral concentrations of Ca, and P during periods of intense shell formation, and egg laying (Waddell, Board, Scott, and Tullett, 1991), and during increased active bone remineralization (Ren *et al.*, 2019). Similar observations were reported by Suchý, Straková, Večerek, and Šterc (2001), and Burnharm, Peebles, Branton, Jones, and Gerard (2003), that plasma mineral concentrations during the egg laying period are influenced by laying rate, and feed energy requirements. According to Sturkie and Mueller (1976), the

Table 4.16: Serum minerals concentration of laying birds fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Calcium (mg/dl)	9.30^{a}	9.30^{a}	9.10^{a}	8.70^{b}	8.50^{b}	0.09
Phosphorus (mg/dl)	3.00^{a}	2.80^{b}	2.60^{c}	2.50^{c}	2.50^{c}	0.05
Sodium (mmol/l)	135.00^{a}	133.00^{ab}	132.00^{bc}	131.00^{bc}	130.00^{c}	0.54
Potassium (mmol/l)	3.60^{a}	3.40^{b}	3.30^{c}	3.00^{d}	3.00^{d}	0.06
Chloride (mmol/l)	102.00^{a}	101.00^{ab}	100.00^{b}	98.00^{c}	96.00^{d}	0.62
Bicarbonate (mmol/l)	26.00^{c}	26.00^{c}	28.00^{b}	28.00^{b}	30.00^{a}	0.41
Serum Ca/P	3.10^{b}	3.32^{a}	3.51^{a}	3.48^{a}	3.40^{a}	0.05

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

blood of a laying hen contains about 20 – 30 mg Ca, but the eggshell formation process requires several times as much Ca, and P than found in the extra-cellular pool (Bar, 2008). Reports suggest that the shell formation process could rapidly drain or use up about 100 -150 mg/hour of ionic calcium in blood within 8 – 18 minutes which must be replenished continuously through intestinal absorption from ingested feed, and mobilization from bone in order to guarantee adequate shell formation (Ren *et al.*, 2019). Therefore, the serum mineral reductions observed in ACAPS treated birds are likely results of increased mineral (Ca and P) utilization for eggshell formation (Ciftci, 2012). This probably led to the continuous, and rapid withdrawal of blood ionic Ca, thus reducing its concentration below the levels observed in the less-laying control birds. Franco-Jimenez and Beck (2005) suggest that the relatively lower laying capacity observed in the control birds, may allow such hen to build up better calcium reserves, and improved calcium utilization from the medullary bone to sustain eggshell quality. Again, it has been reported by Van De Velde *et al.* (1986), that if the time of blood analysis coincides with the time (4 – 22 hours) after the initial oviposition when egg yolk is within the shell gland for shell deposition, serum Ca, and P values will relatively be lower.

Varying ranges of serum ionic calcium have been reported in laying hen. Cerolini Baldi, and Cavalchini (1990), reported a range of 12.01 – 33.18 mg/dl serum Ca concentrations in laying hens of about 18 – 67 weeks of age, while Kurtoglu *et al.* (2007) recorded a relatively lower range (9.9 – 13.76 mg/dl) of Ca, in Brown Nick hens. Therefore, the 8.5 mg/dl (L5) – 9.30 mg/dl (control) obtained in this study compares favourably with the ranges reported for healthy chicken (CDD, 1990). Similarly, the serum P concentrations recorded in this study are same as the mean value (3.2 mg/dl), reported in Warren line hens at 18th week of age (Cerolini *et al.*, 1990; CDD, 1990).

Higher dEB status in layer rations have been observed to significantly decrease the serum chloride concentrations (Gezen *et al.*, 2005), which conforms with our decreasing concentration of serum chloride in the sera of the AC, and ACAPS fed birds. The increase in serum HCO_3^- levels in the AC, and ACAPS group has been associated with increased Na content in rations (Gezen *et al.,* 2005). Other studies have also reported decreased bicarbonate levels in blood with the increment in Na content in layer diets (Austice and Keshavarz, 1984). It is therefore probable that since AC supplementation in the diets increased the Na levels in treated feeds, it may be responsible for the relatively higher bicarbonate levels witnessed in the serum of the treated birds.

Elements such as sodium (Na), potassium (K), and chlorine (Cl) are involved in osmo-regulation, acid–base balance, and transmission of nerve impulses (Ohanaka, 2016). The serum Na values obtained are within the normal range for chicken (Garalevicene, 2003; Mitruka and Rawnsley, 1997; CDD, 1990), while HCO_3^- concentrations across groups are also within the range reported by Sauer *et al*. (2019).

4.7 Faecal Characteristics, and other Environmental Effects of AC and ACAPS Supplementation in Layer Diets

4.7.1 Concentrations of Faecal Minerals

Data representing the mineral content of faeces voided by the experimental laying hens are shown in table 4.17. Generally, the order of abundance of the minerals in the faecal materials was K> P> Na> Ca> Mg> Fe> Mn> Zn> Cu > Cl > N. This mimicked the order of micro mineral concentrations in the layer diets (Table 4.6), while macro minerals such as K, and Na displaced Ca, and Mg from their positions. The results suggest that there are higher concentrations of most of the minerals in the faeces, than the levels found in the diets. For example, the levels of K, P, Na, Mn, Fe, Cu voided in faeces by the control birds were much higher than the respective mineral concentrations in the control diet, with K, and Na concentrations more than doubling. The same trend was observed across AC treated groups for these minerals.

Faecal Ca, Na, and Cl concentrations of treated birds were higher than the control values, which also mimicked the trend in the experimental diets. The concentrations of these elements in the AC treated diets may have exceeded the dietary requirements of the birds thereby leading to their increased concentrations in the excreta. However, blending the AC with aged palm wine resulted in slight lowering of the Ca excretion in L5, and Na excretion in L4, and L5 when compared to L3 diet which had the highest faecal concentrations of these minerals.

Faecal mineral concentrations of K, P, Mg, Fe, Mn, Cu, and Zn decreased significantly ($p<0.05$) below the control values due to the AC, and ACAPS supplementations. The decreasing concentrations of these minerals in the AC, and ACAPS supplemented diets (Table 4.5) may be responsible for these results and has been linked to the adsorption properties of the AC (Wang *et al.*, 2006; Grenier and Applegate, 2013). Ohanaka *et al.* (2018b) also observed decreased mineral excretion due to palm kernel shell ash inclusion in broiler diets.

The hen fed the L4 and L5 diets had lower faecal concentrations of Cu compared to other treated groups, while the hen fed the L4 diet had the least Mg, P, Mn, Zn, and N concentrations in their excreta, which indicates better absorption of these minerals by this group. Okoli *et al*. (2014) reported similar results with pullets fed commercial layer rations supplemented with plantain stalk, and plantain root-based ashes. The study specifically reported reductions in faecal K, Mg, Ni, Fe, and Mn, and concluded that plantain ash is a good source of absorbable minerals, and that it has the potential to improve mineral uptake from commercial diets. These findings indicate that AC, and ACAPS enhance the bioavailability of several minerals in the diet through mechanisms that have not been fully understood. The supply of dietary minerals more than the animal's requirement, decreased the efficient utilization of such minerals leading to higher faecal mineral excretion (Spears, 1996). Excessive mineral contents of animal

Table 4.17: Faecal mineral excretion of laying birds fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Calcium (mg/kg)	3761.67^{d}	3799.23^{c}	4000.49^{a}	3981.42^{a}	3930.87^{b}	26.10
Magnesium (mg/kg)	3113.80^{a}	2897.55^{b}	2707.82^{d}	2693.21^{d}	2813.75^{c}	41.09
Potassium (mg/kg)	14908.92^{a}	14605.62^{b}	14046.96^{d}	14182.78^{c}	14154.83^{c}	87.37
Phosphorus (mg/kg)	7958.21^{a}	6333.77^{b}	6017.08^{c}	5801.82^{e}	5939.99^{d}	212.15
Sodium (mg/kg)	3850.53^{b}	4125.68^{b}	5363.81^{a}	4702.86^{ab}	4798.24^{ab}	181.50
Manganese (mg/kg)	171.42^{a}	148.12^{b}	138.45^{d}	136.25^{e}	141.29^{c}	3.42
Iron (mg/kg)	809.68^{a}	530.60^{b}	399.32^{c}	401.89^{c}	406.70^{c}	42.28
Copper (mg/kg)	15.19^{a}	12.95^{b}	12.87^{b}	12.25^{c}	12.22^{c}	0.29
Zinc (mg/kg)	54.70^{a}	42.20^{b}	38.80^{c}	38.01^{d}	39.26^{c}	1.66
Chlorine (mg/kg)	8.38^{c}	8.85^{b}	9.23^{a}	8.96^{b}	9.15^{a}	0.08

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

manure have been shown to cause soil toxicity, and groundwater pollution when used as fertilizer in crop production (Onweremadu, Okoli, Emenalom, Opara, and Eshet, 2006). Therefore, the reductions in mineral excretions recorded in this study may have some environmental benefits since the faecal materials will serve as eco-friendly manure when applied to the soil as fertilizer.

4.7.2 Effect of AC and ACAPS supplementation on faecal litter and eco-characteristics

Table 4.18 shows the results on the effects of AC and ACAPS supplementation on the faecal moisture, crude protein, odour, and fly infestation. The moisture levels were within the 10-12% range needed to discourage fungal growth in such substrates. The L2, and L3 (AC treated group) values however differed significantly ($p<0.05$) from the control, and the L4 and L5 (ACAPS treated) groups.

The faecal materials contained high levels of crude protein (20.07 - 23.51%), with the AC, and ACAPS treated groups recording significantly higher ($p<0.05$) values than the control. This is probably due to the binding of nutrients such as proteins, and fat by the AC, and resulting in the birds voiding them with the faeces (Osol, 1975; Wang *et al.*, 2006). The high crude protein content which also translates to high nitrogen content indicates that the faeces from these birds will serve as excellent fertilizer for crops. The lower odour and fly infestation recorded in the study among the AC and ACAPS treated birds have also been reported by Durunna *et al.* (2018) in broilers fed diets supplemented with wood charcoal. The AC supplementation of diets could therefore be used to improve the air quality, and control flies in poultry farms.

Table 4.18: Effect of AC and ACAPS supplementation on faecal moisture, crude protein, odour and fly infestation

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Faecal moisture	10.62^b	9.03^c	12.73^a	10.95^b	10.81^b	0.32
Faecal crude protein (%)	20.07^d	23.18^b	23.51^a	22.93^c	23.24^b	0.34
Odour	High	Low	Low	Low	Low	-
Fly infestation	+	-	-	-	-	-
Nitrogen (%)	3.21^d	3.71^b	3.76^a	3.67^c	3.72^b	0.05

4.8 Reproductive Organs, and Hormonal Characteristics of the Laying Hen Fed AC and ACAPS Supplemented Diets

4.8.1 Reproductive hormonal profiles of the laying hen fed AC, and ACAPS supplemented diets

The reproductive hormonal profiles of the laying hens fed AC, and ACAPS supplemented diets are shown in table 4.19. The variations in plasma luteinizing hormone (LH), and follicle stimulating hormone (FSH) concentrations were not significant among the groups. The LH values however decreased numerically, and progressively below the control level due to increasing levels of AC supplementation in the diets, while the increasing levels of ACAPS supplementation resulted in progressive increase above the control level. The AC, and ACAPS supplementations also increased the circulating plasma oestradiol (E2) in the L3, and L4 birds respectively beyond the levels recorded in the other groups, with their values being significantly higher ($p<0.05$) than the L5 values. The birds on the L5 diet however recorded significantly higher progesterone (P4) concentration than all the other groups which also had similar values ($p>0.05$) with the control group.

Aro (2019) reported much higher levels (434.07 - 1876.70 pg/ml) of E2 in late laying hens fed graded levels of dietary salt during which period their percentage hen-day production ranged from 42.70 – 50.75. Williams and Harvey (1986) published much lower E2 values (26 – 364 pg/ml) than the present results as the normal range for female chicken. The LH, and FSH values were also higher at ranges of 0.30 – 15.80 iu/ml and 3.34 – 34.53 iu/ml respectively. According to William and Harvey (1986), the normal range for LH in the female chicken is 1.75 – 3.38 iu/ml, while Bioassay Technology Lab reported 0.2 – 60 iu/ml for FSH. The LH values recorded in this study are therefore lower than the reference range, while the FSH are within the range. The hormonal levels recorded in the present study however supported much higher percentage hen-day production than reported by Aro (2019), indicating a better interplay

Table 4.19: Hormonal assay of laying hen fed AC and ACAPS supplemented diets

Parameters	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Luitenizing hormone (mIU/mL)	0.175	0.122	0.100	0.238	0.380	0.02
Follicle stimulating hormone (mIU/mL)	0.360	0.337	0.360	0.350	0.380	0.02
Estradiol (pg/mL)	730.95 ^{ab}	714.05 ^{ab}	776.00 ^{a}	829.90 ^{a}	582.05 ^{b}	28.46
Progesterone (ng/mL)	0.287 ^{b}	0.527 ^{b}	0.487 ^{b}	0.147 ^{b}	1.063 ^{a}	0.10

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

of the hormones in follicular development and ovulation. Interestingly, the L5 group that recorded significantly low E2 and high P4 ($p<0.05$) also recorded significantly high percentage hen-day production (Table 4.7). Indeed, the results show that progressive increase in E2 concentration corresponded with increasing percentage hen-day production values.

Reproductive hormones such as GnRH, LH, FSH, E2, and P4 simultaneously regulate ovarian follicular development, and ovulation which consequently impacts on egg formation, and rate of lay in domestic hen (Hernandez and Bahr, 2003; Long *et al.,* 2016). The serum concentrations of these hormones in the laying hen are therefore considered useful predictors of their egg laying performance (Lewis *et al.,* 2005; Mohammadi and Ansari-Pirsaraei, 2014). Studies have shown that FSH is particularly involved in the recruitment of small yellow follicles (SYF) into the ovarian hierarchy, and their rapid growth, and maturation (Li and Johnson, 1993).

4.8.2 Reproductive tracts development in laying hen fed AC, and ACAPS supplemented diets

Data representing the reproductive tract morphology of laying hen fed AC, and ACAPS supplemented diets are shown in Table 4.20. There were no significant treatment effects on the liveweight of birds, intact reproductive tracts, and weights of the residual ovary. The ACAPS supplementation resulted in significantly higher ($p<0.05$) ovarian, and large yellow follicles (LYF) weights than the 1.00% AC supplementation, indicating that aged palm wine was responsible for the enhanced effects at that level of AC supplementation. Oviductal length however, reduced significantly ($p<0.05$) in response to the ACAPS supplementation, while oviduct weight reduced only at L5 level of supplementation. The differences in values across groups were due to the size and number of large yellow follicles (LYF) within the ovarian hierarchy of each group (Renema, Robinson, Proudman, Newcombe, and Mckay, 1999). Aro (2019) however published similar intact reproductive tract and ovarian weights to the values

Table 4.20: Reproductive tract development in laying hen fed AC and ACAPS supplemented diets

Parameter	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Live weight (g)	1606.67	1773.33	1793.33	1583.33	1783.33	37.69
Intact reproductive tract weight (%)	6.58	5.94	7.63	6.37	6.78	0.29
Weight of ovary (%)	1.74^{ab}	1.66^{ab}	1.39^{b}	1.90^{a}	1.97^{a}	0.08
Weight of residual ovary (%)	0.29	0.32	0.26	0.30	0.24	0.01
Weight of large yellow follicles (%)	1.40^{ab}	1.51^{a}	1.05^{b}	1.59^{a}	1.66^{a}	0.08
Weight of oviduct (%)	3.40^{ab}	3.29^{ab}	3.39^{ab}	4.43^{a}	2.98^{b}	0.20
Oviduct length (cm)	80.00^{ab}	78.00^{abc}	81.00^{a}	67.00^{c}	69.00^{bc}	2.01

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

recorded in this study. From our observation, the L1, and L3 groups had fewer numbers of LYF that are in the terminal growth phase, which consequently impacted on their hierarchal follicle weights. On the other hand, L5 had larger ovarian follicles sizes within its hierarchy, and more of the follicles in sets of similar size.

The interplay of gonadotropic hormones (FSH and LH) influences the growth, and maturation of follicles, thus increasing their number and size. These follicles rapidly accumulate lipids, and proteins that are synthesized in the liver. Supplementation of the layer rations particularly with 1.00% ACAPS (L5), 0.50% ACAPS (L4), and 0.5% AC (L2), seems to increase the maturation of the Graafian follicles ready to ovulate compared to L3, and the control.

Laying performance, is determined by the rate of development, and maturation of follicles within the hierarchy in the ovary (Meng *et al.*, 2013; Bulbul *et al.*, 2015). This is supported by the percentage hen-day production results in table 4.8 in which the best performance results were recorded in the L5, L4, L3, and L2 groups in that order. The reduced weight of residual ovary in the L5 group despite having the greatest mass of hierarchal follicles may explain in part the reductions in plasma E2 concentration witnessed in this group. The large numbers of LYFs in its ovaries produced more P4, and therefore suppressed the secretion of E2 (Renema *et al.*, 1999; Buchanan *et al.*, 2001).

4.8.3 Number of ovarian follicles of laying hen fed AC, and ACAPS supplemented diets

Table 4.21 highlights the data on the number of ovarian follicles in the laying hen fed the AC, and ACAPS supplemented diets. The number of large yellow follicles increased with the dietary treatment level such that the L4 birds fed 0.5% ACAPS recorded significantly higher ($p<0.05$) numbers of LYF than all the other groups. The 1% ACAPS (L5) birds recorded similar LYF numbers with the AC treated group (L2 and L3) while the control group recorded the least number of LYF. A similar trend was observed with the small yellow follicles (SYF).

The development of ovarian follicles involves the maturation, and recruitment of follicles into the yolk-filled hierarchy (F1-F6). The F1 or Graafian follicle is the largest LYF in the hierarchy, while the last follicle or (F5/F6), within the sequence is the smallest LYF. At ovulation, the F1 dehisces, and releases ovum into the oviduct during the egg formation process, while another follicle or F2 matures, and becomes the new F1, and so on, thus sustaining daily egg production in the chicken. The increased number of LYFs particularly in the L4, and L5 may be responsible for the sustained egg production in these groups compared to the control as shown in table 4.8.

The L4 birds also had a significantly higher ($p<0.05$) number of pre-hierarchal or small yellow follicles (SYF) followed closely by 0.5% AC group (L2). Birds fed the L3 and L5 diets had the same number of SYF which were significantly lower than L4 and L2 values, while the control had the least number of SYF.

Again, all the AC, and ACAPS treated birds had higher numbers of large white follicles compared to the control. The L4 birds had a greater number of large white follicles (LWF) similar to L2, L3, and L5 and were significantly higher than the values recorded in the control. The SYF, and LWFs provide a constant supply of growing follicles for the hierarchy (Gilbert *et al.*, 1983), and these developed follicles form the large yellow follicles (LYF) in the ovary. Higher numbers of these follicles will influence rate of egg laying, while lower numbers as found in the control will increase the frequency of pause day without any egg laid, and consequently decrease rate of egg production. The feeding of AC, and ACAPS diets did not significantly increase the number of small white follicles in the ovary. However, L2 maintained a higher numerical number, while the control birds had the least value.

Table 4.21: Number of follicles in laying hen fed AC and ACAPS supplemented diets

Parameter	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Numbers of LYF (≥ 10 mm)	4.50 ^{c}	5.00 ^{bc}	5.00 ^{bc}	7.00 ^{a}	6.50 ^{b}	0.32
Numbers of small yellow follicle (≥5-9 mm)	4.67 ^{c}	11.00 ^{ab}	8.67 ^{bc}	14.50 ^{a}	7.00 ^{bc}	1.05
Numbers of LWF (≥ 4-6 mm)	3.50 ^{b}	5.50 ^{ab}	7.00 ^{ab}	9.00 ^{a}	6.50 ^{ab}	0.68
Numbers of small white follicle (≤ 3 mm)	11.00	20.50	18.33	13.00	16.50	1.93

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

4.8.4 Characterization of Ovarian follicle sizes of laying hen fed AC, and ACAPS supplemented diets

The hierarchal follicle dimensions of laying hen fed diet supplemented with AC and ACAPS is presented in Table 4.22. No significant treatment effects were recorded on the follicle dimensions because of the supplementations in the layer rations except in the pre-ovulatory follicles within the range of 10-14 mm. The L2 and L4 birds had the largest F4 preovulatory follicles which were also like the L3 and L5 follicles values. Aro (2019) also reported significant increases in the numbers of preovulatory follicles (10-14 mm) in laying hen fed varying levels of dietary salt. The selection of follicles based on dimensions is useful for the determination of those that will undergo further development to final ovulation.

4.9 Physico- Chemical Characteristics of the AC Supplemented Broiler Diets

4.9.1 Physical characteristics of the starter and finisher diets

Broiler starter: The results of the physical characteristics of the broiler starter diets are summarized in Table 4.23a. The bulk density (BD), and specific gravity (SG) values of the starter diets decreased because of the dietary AC supplementation, with the B2 values being significantly different ($p<0.05$) from the control values. The water holding capacity (WHC) followed a reverse trend with B2 value also being significantly lower ($p<0.05$) than others. Omede (2010), and Alaoma (2016) reported BD values of 0.29 – 0.46 g/cm^3, and 0.584 – 0.672 g/cm^3 respectively for major commercial broiler starter diets produced in Nigeria, indicating that the experimental diets were denser than these commercial diets. This could also mean that the ingredients used in formulating the experimental starter diets had low NSP or fibre components, and were of uniform small particle size, and therefore had reduced tendency to segregate or separate in the final feed (Omede, 2010; Iwuji *et al.*, 2013). Smaller sized particles

Table 4.22: Follicular dimensions of LYF in laying hen fed AC and ACAPS supplemented diets

Parameter	L1 Control	L2 0.50% AC	L3 1.00% AC	L4 0.50% ACAPS	L5 1.00% ACAPS	SEM
Mature follicle (≥25 mm)	29.50	29.00	30.00	30.00	30.17	0.36
20-24 mm	22.25	23.00	22.50	23.25	23.17	0.16
15-19 mm	16.00	17.00	16.25	16.50	16.00	0.58
10-14 mm	10.50^{b}	11.25^{a}	10.75^{ab}	11.33^{a}	11.00^{ab}	0.11

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Table 4.23: Physical characteristics of experimental broiler feeds

(a) Starter

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	SEM
Bulk density (g/ml)	0.839^{a}	0.808^{b}	0.820^{ab}	0.01
Water holding capacity (%)	131.53^{b}	127.99^{c}	134.90^{a}	1.03
Specific gravity	0.879^{a}	0.823^{c}	0.847^{b}	0.01
pH	6.48^{a}	6.24^{b}	6.59^{a}	0.05
(b) Finisher	B1	B2	B3	SEM
Bulk density (g/ml)	0.782^{a}	0.739^{b}	0.741^{b}	0.01
Water holding capacity	134.19^{a}	126.61^{c}	128.30^{b}	0.59
Specific gravity	0.819^{a}	0.748^{b}	0.760^{b}	0.01
pH	6.65	6.58	6.66	0.04

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

have higher bulk densities than larger sized ones (Fasina, 2006), in that larger particles possess increased pore volume compared to smaller particles (Mani *et al.*, 2004).

The SG values are higher than the range of 0.33 – 0.46 reported by Omede (2010) but fell within the range reported by Alaoma (2016), for commercial broiler starter rations produced in Nigeria. Low SG feed have low retention time, and faster rate of feed transit within the GIT (Bhatti and Firkins, 1995). Again, the higher WHC value recorded in B3 diet still relates to its increased absorptive surfaces due to the increased AC supplementation. Nevertheless, the WHC values are comparable to the range reported for commercial broiler starter diets produced in Nigeria (Omede, 2010; Alaoma, 2016). Generally, the pH values obtained for the starter broiler diets are within the range reported for broiler diets (Brzoska, Śliwiński, and Michalik-Rutkowska, 2013).

(b) Broiler finisher diets:

The control finisher diet recorded significantly higher ($p<0.05$) values of the BD, WHC, and SG, indicating treatment effects of the AC supplementations. The trend of the WHC results was reversed in the finisher diets, when compared with the starter diets indicating that AC supplementation reduced the ability of the diets to hold water. The starter diets were expectedly denser than the finisher diets, while pH was generally mildly acidic across feed types. The BD, and SG values of the finisher diets are however slightly higher than the range reported for commercial finisher feeds produced in Nigeria, while WHC values were within the reported range (Omede, 2010; Alaoma, 2016).

4.9.2 Proximate compositions of the starter and finisher diets

(a) Broiler starter

The proximate compositions of the broiler starter diets supplemented with AC are represented in Table 4.24a. The crude protein (CP), crude fibre (CF), and total ash (TA) contents of the diets decreased because of the AC supplementation such that the B2, and B3 diets recorded significantly lower ($p<0.05$) values than B1. This could be as a result of nutrient dilution, and adsorptive characteristics of the activated charcoal (Wang *et al.,* 2006). The nitrogen free extract (NFE) increased with increasing AC supplementation such that the control value was significantly lower ($p<0.05$) than the B2 and B3. The moisture content values are within the acceptable limit reported for broiler starter diets produced in the tropics (Okoli *et al.,* 2007; Uchegbu *et al.,* 2008; Alaoma, 2016), and therefore will not support fungal growth.

Interestingly, B3 (1% AC addition in feed) recorded significantly higher ($p<0.05$) crude fat and metabolizable energy (ME) values than the control, and B2 diets. Generally, the CP, CF, ash, and crude fat contents of the experimental diets are within the recommended values for optimal broiler production in the tropics (Okoli *et al.*, 2007; Alaoma, 2016; SON, 2018). The 3022.49 Kcal recorded in B3 is also within the values recommended for Abor Acre broiler starter diets (Aviagen, 2019; SON, 2018), while the rest are below the optimal value, and therefore may impact the growth performance of the birds negatively.

Table 4.24: Proximate composition of experimental broiler diets

(a) Starter diet

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	SEM
Moisture (%)	10.51^{b}	11.30^{a}	10.69^{b}	0.15
Crude protein (%)	21.64^{a}	20.89^{b}	19.23^{c}	0.36
Crude fat (%)	3.48^{b}	2.71^{c}	5.02^{a}	0.34
Crude fibre (%)	6.58^{a}	6.38^{b}	6.23^{c}	0.50
Total ash (%)	15.30^{a}	14.27^{b}	13.77^{c}	0.23
Nitrogen free extract (%)	42.39^{b}	44.67^{a}	45.15^{a}	0.43
Metabolizable energy (kcal/kg)	2910.40^{b}	2870.33^{c}	3022.49^{a}	22.84

(b) Finisher diet	B1	B2	B3	SEM
Moisture (%)	9.61	9.60	9.47	0.04
Crude protein (%)	20.85^{a}	19.21^{b}	19.08^{c}	0.29
Crude fat (%)	6.87^{a}	5.95^{b}	5.67^{c}	0.18
Crude fibre (%)	5.11^{a}	4.79^{b}	4.60^{c}	0.08
Total ash (%)	15.05^{a}	14.29^{b}	14.03^{c}	0.15
Nitrogen free extract (%)	42.52^{c}	46.17^{b}	47.17^{a}	0.71
Metabolizable energy (kcal/kg)	3147.69^{b}	3153.89^{b}	3175.39^{a}	4.50

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

(b) Broiler finisher diets

Both the CP, CF, crude fat, and TA values decreased progressively with increasing AC supplementation, such that the control group values were significantly higher ($p<0.05$) than the AC supplemented group values. The NFE, and ME values followed the reverse trend, while there were no significant differences among the groups in moisture content. These results confirm the hypothesis that AC may adsorb or desorb not only toxins, drugs, and pathogens, but also useful nutrients when added in the feed (Osol, 1975; Plank *et al.,* 1990; Kutlu *et al.,* 2001). Like the results of the broiler starter diets, AC supplementation, increased the NFE, and metabolizable energy components of the diets. Optimal ME values of 3100- 3200 kcal/kg have been recommended for finisher broiler diets (SON, 2018; Aviagen, 2019). Thus, the range values of 3147.69 – 3175.39 ME kcal/kg obtained in this study suggest that all the diets will support optimal broiler performance at the finisher stage of development.

4.9.3 Mineral concentrations in the broiler starter and finisher diets

The mineral concentrations of the broiler starter, and finisher diets are summarized in tables 4.25 a and b respectively.

(a) Broiler starter diets

The most abundant macro minerals in the starter diets were calcium, potassium, phosphorus, sodium, and magnesium, while for the micro–minerals, they were iron, manganese, zinc, copper, and chlorine. Thus, the order of mineral abundance was Ca> K> P> Na>Mg> Fe> Mn> Zn> Cu>Cl.

There was a progressive reduction in the dietary concentrations of Ca, Na, and Cl in AC treated diets, while K, P, Fe, Mn, Cu, and Zn increased compared to the control values. These findings are contrary to the results of macro and micro - mineral concentrations in the layer diets (Table

4.6) in which K, P, Mg, and Fe, Mn, Cu, and Zn concentrations decreased with increasing AC supplementation in the diets. Similar decrease in dietary concentrations of Ca, K, and P was also reported by Ohanaka, (2016) when palm kernel shell ash was supplemented in broiler diets particularly at higher dietary inclusion levels.

The values obtained in this study however met, and surpassed the minimum Ca, P, and K concentrations recommended for broiler starter rations (SON, 2018; Aviagen, 2019). The P concentration in the AC supplemented diets were exceptionally high, especially in B3, and may relate to the P concentration in the pig dung used in the AC production which has been reported to increase P concentration in ACs (Lima and Marshal, 2007). The K concentrations were slightly higher than the 5000 mg/kg recommended for broiler starter diets (Rostagno *et al.,* 2000). According to Johnson and Farrell (1985), feeding birds with diets containing higher levels of K will cause reductions in feed intake, and FCR due to alterations in their energy metabolism.

The Na, Ca, and all the micro-mineral concentrations were within the levels recommended for broiler starter diets (NRC, 1994; SON, 2018). The Cl, Mn, Cu, and Zn values were however below the recommended levels for broiler starter diets by Aviagen (2019), while the Fe requirements were generally surpassed in all the diets.

(b) Broiler finisher diets

In the finisher diets (Table 4.25b), the trend of abundance in mineral concentrations was like those reported for broiler starter diets. Thus, the order of mineral abundance in the finisher diet was Ca> K> P> Na> Mg> Fe> Mn> Zn> Cu> Cl. The concentrations of Ca, Na, and Cl in the AC supplemented diets also decreased ($p<0.05$) below the control values. The other macro, and micro -minerals also followed the same trend of increase with AC supplementation reported in the starter diets, although they remained within the values

Table 4.25: Mineral composition of experimental broiler diets

(a) Starter diet

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	SEM
Calcium (mg/kg)	12309.23^{a}	12168.62^{b}	12013.71^{c}	34.23
Magnesium (mg/kg)	1544.51	1709.68	1790.74	121.30
Potassium (mg/kg)	5220.27^{c}	6211.97^{b}	6846.02^{a}	236.54
Phosphorus (mg/kg)	4503.57^{c}	7334.52^{b}	9652.20^{a}	744.37
Sodium (mg/kg)	2642.59^{a}	2119.77^{b}	1981.84^{c}	100.62
Manganese (mg/kg)	48.42^{c}	53.16^{b}	76.45^{a}	4.33
Iron (mg/kg)	316.09^{c}	361.96^{b}	453.33^{a}	56.11
Copper (mg/kg)	9.13^{c}	10.03^{b}	12.95^{a}	0.58
Zinc (mg/kg)	40.76^{c}	47.54^{b}	52.52^{a}	1.70
Chlorine (mg/kg)	12.97^{a}	10.99^{c}	12.57^{b}	0.31
(b) Finisher diet	B1	B2	B3	SEM
Calcium (mg/kg)	10701.24^{a}	10197.77^{b}	9867.06^{c}	121.30
Magnesium (mg/kg)	1578.86^{c}	1855.70^{b}	2195.47^{a}	89.16
Potassium (mg/kg)	4879.68^{c}	5179.57^{b}	5895.65^{a}	150.69
Phosphorus (mg/kg)	3201.34^{c}	4101.10^{b}	5198.67^{a}	288.76
Sodium (mg/kg)	2048.12^{a}	1670.16^{b}	1390.57^{c}	95.26
Manganese (mg/kg)	41.61^{c}	50.07^{b}	59.78^{a}	2.63
Iron (mg/kg)	287.68^{c}	304.36^{b}	371.07^{a}	12.74
Copper (mg/kg)	9.17^{c}	11.11^{b}	13.28^{a}	0.59
Zinc (mg/kg)	31.88^{c}	38.72^{b}	41.70^{a}	1.45
Chlorine (mg/kg)	12.21^{a}	9.59^{c}	11.42^{b}	0.39

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

recommended by SON (2018) for broiler finisher diets. The reduction in Na concentration resulting from 1% AC supplementation however resulted in a value below the 1600-2000 mg/kg recommended by Aviagen (2019). The iron, manganese, zinc, and copper concentrations also increased significantly ($p<0.05$) above the control values and were generally higher than the recommended values for finisher broiler rations produced in Nigeria (SON, 2018), except the Zn concentrations.

4.9.4 The mineral ratios, and dietary electrolyte balance of the AC supplemented diets

The mineral ratios, and dietary electrolyte balance of the broiler starter, and finisher diets were summarized in tables 4.26 a and b respectively.

(a) Broiler starter diets

The Ca/P ratios of the starter diets decreased progressively with increasing inclusion of AC in the diets, such that the control value was significantly higher ($p<0.05$) than the values of the AC treated diets. The Na/K ratios followed similar trend, although values were not significantly different ($p>0.05$). The variations in the Ca/P ratios obtained were probably because of the increasing concentration of P, especially in the AC treated diets which were much higher than the recommended limits for broilers at early developmental stage. Ca/P ratios of 2:1 or 1:1 with a mean available P of 0.5%, have been reported adequate for normal bone formation in growing chicks (NRC, 1994; Li, Zhang, and Bryden, 2017; Aviagen, 2019), indicating that the ratios obtained in this study are within the recommended ratios.

The dEB values increased significantly with increasing AC supplementation levels in the diets, such that the control diet recorded significantly lower ($p<0.05$) dEB values than the others. Szabo *et al.* (2011) suggested a dEB of 175 Meq/kg as appropriate for starter broilers, while a dEB range of 246 - 315 Meq/kg has earlier been suggested (Oviedo-Rondon, Murakami, Furlan, Moreira, and Macari, 2001; Murakami, Oviedo-Rondo, Martins, Pereira,

Table 4.26 Mineral ratios and the dietary electrolyte balance of the experimental broiler diets

(a) Starter diet

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	SEM
Ca/P ratio	2.73^{a}	1.66^{b}	1.25^{c}	0.22
Na/K ratio	0.51	0.34	0.29	0.03
dEB (Meq/kg)	248.38^{c}	251.14^{b}	261.35^{a}	1.97

(b) Finisher diet	B1	B2	B3	SEM
Ca/P ratio	2.96^{a}	1.97^{b}	1.67^{b}	0.22
Na/K ratio	0.42	0.32	0.24	0.03
dEB (Meq/kg)	213.83^{a}	205.16^{c}	211.31^{b}	1.29

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

and Scapinello, 2001). A more recent study by Unamba-Oparah *et al.* (2017) reported a dEB range of 137.5 – 262.06 Meq/kg for major commercial broiler starter diets produced in Nigeria, indicating that values recorded in this study are within the range required for optimal broiler performance in the hot tropical environment.

(b) Broiler finisher diets

The Ca/P, and Na/K ratios of the finisher broiler diets followed similar trends as the starter diets, with Ca/P ratio values being significantly lower ($p<0.05$) in AC treated diets than in the control. Again, the concentrations of P, which were higher, and more adequate in AC supplemented diets accounted for these variations. Specifically, the B2 diet had similar Ca/P ratio with the values recommended by Aviagen (2019), while the B1, and B3 values were slightly higher, and lower than required respectively. The Na/K ratios of the experimental diets were all within the range recommended by Aviagen (2019).

The dEB status of the control diet was significantly higher ($p<0.05$) than the values recorded in the AC supplemented diets. The dEB values were however generally lower than those recorded in the broiler starter diets. Several studies have suggested dEB range values of 249-257 Meq/kg (Murakami *et al.*, 2001; Oviedo-Rondon *et al.*, 2001), and 250 Meq/kg (Szabo *et al.*, 2011) as suitable for broilers at the finisher stage. However, Borges *et al.* (2003) demonstrated that dEB levels of 236, and 207 mEq/kg, optimized maximum body weight gain, and feed conversion ratio respectively in the finisher broilers under hot summer conditions, while further recommending a dEB range of 202-235 MEq/kg under normal ambient temperatures (Borges *et al.*, 2004). The dEB range recorded in this study is therefore within the recommended range for broiler finisher diets. The feed may however fail to optimize performance, especially under the high temperature conditions witnessed during the later stage of the experiment.

4.10 Growth Performance of Broilers fed AC and ACAPS Supplemented Diets for 28 Days (Starter Phase)

4.10.1 Seventh day performance characteristics

Several reports have consistently suggested the importance of the first seven days of broiler chicken's life. Over the years, the cropping age of broilers has reduced from nine weeks to six weeks, indicating that the first seven days of life have become more important in the overall growth period of the birds. Its influence on the overall performance of the bird as recently reported (Mahapatra, Srinivasan, Rajini, and Mangala, 2017; Ohanaka *et al.,* 2018a) shows the need to monitor growth performance parameters during this period.

(a) Growth performance

Table 4.27 shows that at the end of seven days of feeding, the birds fed the 0.5% AC (B2) recorded the best final weight, and weight gains, and were followed by the control, and B4 (0.5% ACAPS) groups. B3 and B5 (1.0% AC and ACAPS respectively) recorded significantly lower ($p<0.05$) values than B2 indicating that the birds did not tolerate the 1.0% AC inclusion well at that stage of development. The birds on B4 diet however ate significantly more ($p<0.05$) than the groups on B3 and B5 diets, while FCR remained statistically similar across groups. Evans *et al.* (2017) revealed that supplementation of 2% broiler litter-based biochar in one-day-old broiler chicks' diet had no adverse effect on the chicks, and resulted in similar weight gain, and FCR compared with control feed. Higher dietary inclusion levels of AC have been reported to cause discoloration of feed and might also reduce feed acceptability/palatability thereby causing decreased consumption of such feed (Jindal *et al.,* 1994; Kutlu *et al.,* 2001).

Again, AC as an unspecific adsorbent has been shown to not only adsorb noxious substances, toxins, and pathogens in the feed, but may also adsorb useful nutrients such as

Table 4.27: Seventh day performance characteristics of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Initial weight (g)	40.15	40.07	40.00	40.20	39.93	0.09
Final weight (g)	123.04^{ab}	128.35^{a}	112.37^{b}	123.47^{ab}	112.12^{b}	2.38
Weight gain (g)	82.78^{ab}	88.28^{a}	72.37^{b}	83.27^{ab}	72.19^{b}	2.33
Average daily weight gain	11.83^{ab}	12.61^{a}	10.34^{b}	11.90^{ab}	10.31^{b}	0.33
Average daily feed intake (g)	14.27^{ab}	14.58^{ab}	12.34^{c}	15.46^{a}	13.58^{bc}	0.35
FCR	1.21	1.16	1.21	1.32	1.31	0.03

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

vitamins, fats, and enzymes, especially when fed at higher dietary levels, and thus can interfere with digestion (Osol, 1975; Plank *et al.,* 1990; Fujita *et al.,* 2012; Prasai *et al.,* 2017). A recent study by Chikwangwan, (2019) observed significant reduction of coliforms in the digestive tract of broilers during the early developmental stages and concluded that higher (1 – 2%) biochar feeding reduced the population of beneficial microbes which affected nutrient digestion, and absorption, consequently depressing performance in broilers. The proximate, and mineral compositions of the starter diets (Tables 4.24a and 4.25a respectively) showed that higher AC inclusion decreased considerably the CP content, and some essential minerals, indicating that the reduction in growth parameter values observed in the birds on B3 and B5 diets may be due to the reductions in these essential nutrients, and feed intake. Kana *et al.* (2011) also reported slight reduction in feed intake, final weight, and body weight gain when maize cob, and wood-based AC were fed to broilers beyond 0.6%.

Generally, the feed intake, and weight gain results in all groups were below the recommended values by Aviagen (2019) for Abor Acre broilers. The control birds ate 32.50% less feed and gained 23.10% less weight from the 160 g weight recommended for broilers at this age. The AC, and ACAPS treated birds also ate (31.03 – 41.63%) and (26.87 –35.76%) less feed and gained (19.78 – 29.77%) and (2.83 – 29.93%) less weight than the recommended feed intake, and weight gain respectively. Islam (2000), and Ukwu (2013) reported decreased performance in broiler raised under stressful environment and conditions such as hot-humid climate, early feed and water restrictions, poor transportation, and handling practices.

(b) Development of the gastro-intestinal tract (GIT), and the liver

The first week after hatch is a critical period in the life of a broiler chick in terms of digestive efficiency, growth, and sustenance of life (Willemsen *et al.,* 2008). This is because the post-hatch chicks have limited digestive, and immune system functions required for adequate utilization of exogenous nutrients (primarily carbohydrate, and protein-based), and therefore must rapidly undergo substantial physical, and functional GIT, and organ development as well as the development of active immunity (Uni, Noy, and Sklan, 1999; Ukwu, 2013). The chicken's anatomical structure such as the proventriculus, gizzard, small, and large intestines are crucial to their feed conversion efficiency (De Verdal *et al.*, 2010). According to several reports (Sklan, 2001; Ukwu, 2013; Ohanaka *et al.*, 2018a), intestinal growth represents most of the body weight increase at this early developmental stage relative to body weight gain.

All the organ weight parameters (Table 4.28) evaluated at the end of 7 days of feeding showed treatment effects except for the percentage crop weight that was similar in all the groups. Birds on B2 (0.5% AC), and B4 (0.5% ACAPS) diets had similar but significantly higher ($p<0.05$) liveweights than B1 (0% AC), B3 (1% AC), and B5 (1% ACAPS). The control birds also had significantly higher liveweights than the birds fed the B3 diets.

The percentage proventriculus, and large intestine weights decreased progressively with increasing AC, and ACAPS supplementation such that the control value was significantly higher ($p<0.05$) than the B5 value. Being the glandular stomach, a well-developed proventriculus will have more enzymatic functions, and especially the secretion of hydrochloric acid, and mucus (Aspinall *et al.,* 2015; Alshamy *et al.*, 2018).

The gizzard weights were generally similar except for B2 value, which was significantly higher than the B5 value. The gizzard is the digestive segment where powerful contractions crush ingested feed (Dyce, Sack, and Wensing, 2009). According to Kokoszynski Bernacki, Saleh, Steczny, and Binkowska (2017), the high proportion of gizzard weight in the body of birds suggest better muscle development, capable of improving nutrient absorption. A better developed gizzard to a greater extent affects the performance of the birds (Parsons, Buchanan, Blemings, Wilson, and Moritz, 2006, Pacheco, Stark, and Brake, 2013), while an underdeveloped gizzard reduces the retention time of digesta and elevates its content pH which

Table 4.28: Seventh day gastrointestinal development of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Live weight (g)	128.50^{bc}	135.50^{ab}	116.50^{e}	141.00^{a}	121.00^{cd}	2.63
Crop weight %	0.75	0.93	0.91	0.84	0.62	0.05
Proventriculus weight %	0.97^{a}	0.93^{ab}	0.91^{ab}	0.88^{ab}	0.86^{b}	0.02
Gizzard weight %	4.49^{ab}	4.92^{a}	4.46^{ab}	4.52^{ab}	4.15^{b}	0.10
Small intestinal weight %	4.65^{ab}	4.19^{ab}	3.88^{b}	4.97^{a}	4.55^{ab}	0.16
Large intestinal weight %	1.27^{a}	0.99^{ab}	0.88^{b}	0.86^{b}	0.70^{b}	0.06
Liver weight %	3.84^{bc}	4.51^{ab}	3.88^{bc}	3.64^{c}	4.63^{a}	0.13
Villus height (µm)	177.67^{b}	185.67^{b}	168.67^{b}	209.50^{a}	174.00^{b}	4.70
Crypt depth (µm)	8.00	5.00	4.00	26.00	24.50	3.72

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

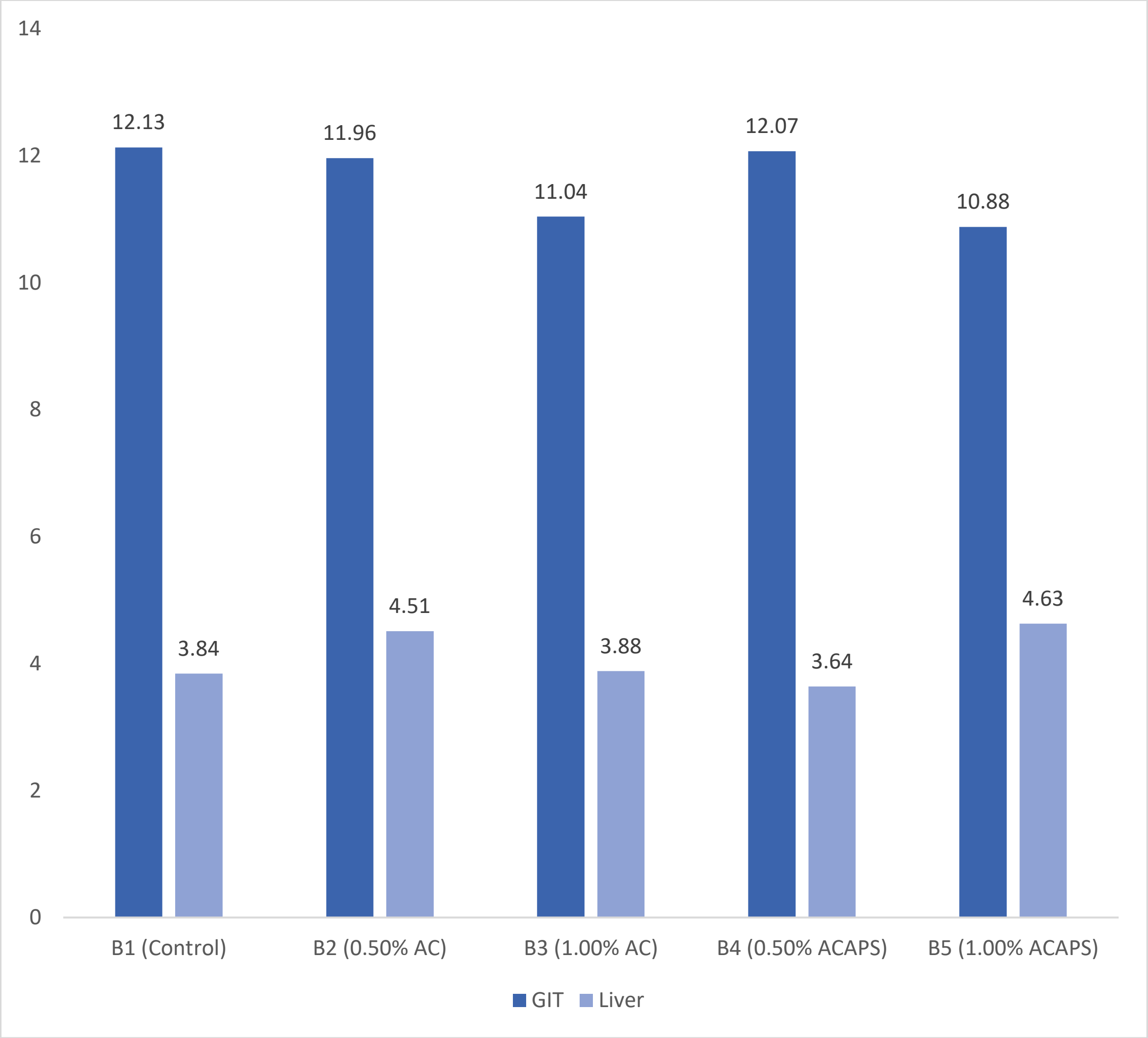

Fig. VI: Percentage GIT and liver weights of broilers after seven days of feeding may result in possible reduction in digestion processes in broilers (Abdollahi and Ravindran, 2013). Therefore, the relatively low gizzard, and proventriculus weights of B5 birds indicates inferior development, and ability to handle the high AC, and ACAPS diets, thus decreasing nutrient absorption.

The percentage small intestinal weight was progressively depressed by increasing AC inclusion level. This depressive effect was however abolished by the ACAPS, such that the B5 value became similar to the control, and B2 value. According to Mabelebele *et al.* (2014), the increase in small intestine weight allows broiler chicks to attain heavier body weight rapidly during early development. The lower percentage small intestine weight of the B3 birds is probably reflective of lower body weight due to reduced feed intake in this group (Ohanaka *et al.*, 2018a). Chicks achieve optimal growth when nutritional requirements are met. Changes in nutrient supply may also have a delayed influence on growth (Noy and Sklan, 1997). Notably, the protein content of the B3 starter diets were reduced below B2, and control diets which may have had consequences on the intestinal development, muscle metabolism, and growth of B3, and B5 birds (Everaert *et al.*, 2010).

Birds on the B1 (0% AC) diet had significantly higher ($p<0.05$) percentage large intestine weight than B3 - B5 groups but similar to B2, while no significant difference ($p>0.05$) in values was recorded between the other groups. The percentage liver weights of birds consuming B5 diet was significantly ($p<0.05$) higher than all the groups but was comparable to that of B2. Birds on the control, and B3 diets also had similar liver weight percentages, while B4 had the least.

Generally, the percentage weights of gizzard, proventriculus, and the small intestine increases rapidly when compared to the bodyweight increases during the early developmental stages of the broiler chicken, to ensure the development of nutrient supply functions necessary for subsequent growth of demand tissues, such as muscle, skeleton, and adipose (Noy and Sklan, 1997; Yadav *et al.*, 2010). Previous measurements by Ukwu (2013), and Ohanaka *et al.* (2018a) showed the superior weight development of these organs over the overall body weight in broilers. Therefore, the reduced weights of these organs witnessed in B5, and B3 should be seen as evidence of inferior GIT development, which consequently affected their growth when compared with the other groups. The overall GIT, and liver weights at the end of seven days of feeding are shown in figure VI. This highlights the effect further, and also shows the relationship between the GIT and liver developments

The result of the intestinal villi development of the experimental birds (plate a - e) showed that the feeding of ACAPS to broilers at the early developmental stage significantly ($p<0.05$) improved the intestinal villi of the birds consuming L4 (0.5% ACAPS) diet, while L5 (1% ACAPS) or AC supplemented groups (L2 and L3) did not influence ($p> 0.05$) villi development more than the control. Similar responses of improved villi height, and cell surface area, have been demonstrated in poultry (Samanya and Yamauchi, 2001; Rattanawut, 2014), fed activated charcoal - wood vinegar supplements in their feed. Increased height of intestinal villi has been associated with more surface area for nutrient absorption (Onderci *et al.*, 2006) leading to improved performance. Invariably, the birds on the B4 (0.50% ACAPS) diet recorded the highest liveweight, percentage small intestinal weight, villus height, and crypt depth values.

The performance effects witnessed with the dietary supplementation of the AC, and ACAPS may be explained as the interactions of the toxin binding, improved digestion, and alteration of intestinal microflora by these additives through the reduction of pathogenic bacteria proliferation as well as increment in beneficial microflora. These benefits are however dose-dependent at the early stage of broiler development with the performance decreasing beyond 0.5% inclusion.

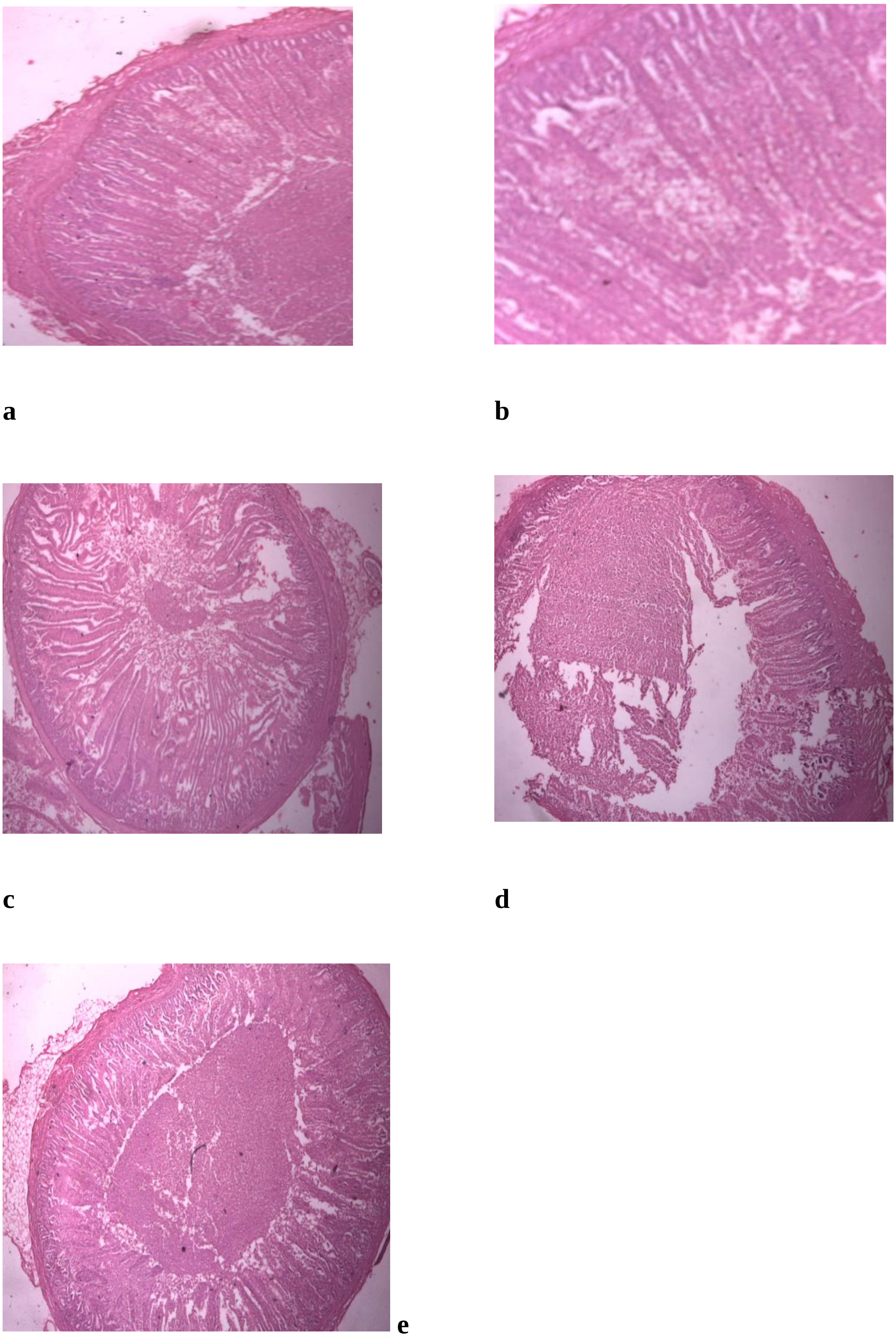

Plate A. Villi histograph of broilers fed AC and ACAPS supplemented diets

(magnification = 400 X)

a = B1 group, b = B2 group, c = B3 group, d = B4 group, e = B5 group

(c) pH of GIT contents

Data representing the pH of GIT contents of the experimental chicks after seven days of feeding are presented in table 4.29. Generally, all the GIT contents pH were slightly acidic except for the gizzard digesta content which was moderately acidic (3.50 – 3.75). Treatment effects were recorded only for the small intestinal content pH values, while the digesta content of crop, proventriculus, gizzard, and large intestine were similar ($p>0.05$) across the groups.

The digesta content pH of the small intestine were higher in B1, B2, B3, and B5 ($p>0.05$) than in B4 ($p<0.05$), while there were no differences ($p>0.05$) between B3, B4, and B5. The more acidic pH of GIT content of the B4 birds, may increase the solubility of calcium, and its absorption in the GIT (Soetan, Akinrinde, and Ajibade, 2010; Ohanaka, 2016).

Several authors have reported the normal small intestinal pH range of broiler chicken to be between 5.7 and 6.5 (Walk *et al.*, 2012; Mabelebele, *et al.*, 2014). Again, at 15 days of age, the digesta content of the duodenum, jejunum, ileum of the small intestine of broilers have been shown to record pH values of 6.4, 6.5, and 8.15 respectively (Rynsburger, 2009).

4.10.2 Growth performance characteristics of starter broilers fed AC and ACAPS supplemented diets

Table 4.30 represents the performance data of chicks subjected to 28 days starter feeding with the experimental diets. The birds fed the B4 (0.50% ACAPS) diet recorded significantly higher final body weight, and weight gains ($p<0.05$) than the birds fed the B3, and B5 (1% AC and ACAPS respectively) diets. The B4 values were however statistically similar to the B1, and B2 values. The average daily feed intake, and FCR values were not statistically different across groups.

Table 4.29: Seventh day pH of GIT content on the seventh day of feeding

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Crop Ph	5.75	6.0	6.0	5.75	6.0	0.05
Proventriculus Ph	4.97	5.25	5.25	5.00	5.50	0.12
Gizzard Ph	3.50	3.50	3.50	3.75	3.50	0.04
Small intestinal pH	6.25^a	6.25^a	6.00^{ab}	5.75^b	6.00^{ab}	0.06
Large intestinal pH	6.25	7.00	6.50	6.50	6.50	0.12

Means with different superscript on the same horizontal row are significantly different @ p<0.05

These results reflect the effects of the GIT development in the birds, especially the small intestine, and villi height results during the first seven days of life. The 0.50% AC, and ACAPS supplementation results tend to agree with earlier reports of improved performance, and no deleterious effects of AC on broilers during the early developmental stage (Shareef *et al.,* 1998; Kutlu *et al.,* 2001; Dim *et al.,* 2018). Wang *et al.* (2006) have suggested that higher AC inclusion may depress the performance of broilers due to nutrient dilution or binding, especially through reductions in energy, and protein availability to the birds with resultant poor performance. This is supported by the proximate composition results (Table 4.24a) in which the 1.0% AC supplemented diets recorded significantly lower crude protein value. The results are also in agreement with several other reports of depressed growth at higher AC inclusion in broiler diets at various developmental stages (Kana *et al.*, 2011; Jiya *et al.*, 2013; ARDCorp, 2014).

4.10.3 Growth performance of finisher broilers fed AC and ACAPS supplemented diets

(a) Six weeks growth performance (4 weeks of starter and 2 weeks of finisher feeding)

The sixth week (42 day) growth performance was evaluated to determine if it will be economical to harvest the birds at that stage of their development. Table 4.31 shows that the birds fed the 0.5% ACAPS diet (B4) recorded significantly higher ($p<0.05$) growth performance values than all the other groups, while feed intake was not significantly different among the groups ($p>0.05$). Other AC, and ACAPS treated diets (B2 B3 and B5) had similar ($p>0.05$) growth performance indices with the control. The B4 group also recorded the superior FCR value, while the control group recorded the most inferior values. These results suggest that the addition of aged palm wine to the 0.5% AC diet potentiated the growth performance effects of the AC. This beneficial effect of the aged palm wine may be attributed to its ability to modify gut pH, and control the proliferation of intestinal microflora, increase the solubility

Table 4.30: Growth performance of starter broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Initial weight	40.15	40.07	40.00	40.20	39.93	0.09
Final weight	654.68^{ab}	670.66^{ab}	616.83^{b}	695.89^{a}	609.70^{b}	11.47
Weight gain	614.53^{ab}	630.60^{ab}	576.83^{b}	655.69^{a}	569.76^{b}	11.45
Average daily weight gain	21.95^{ab}	22.52^{ab}	20.60^{b}	23.42^{a}	20.35^{b}	0.41
Average daily feed intake	36.02	34.10	33.12	36.38	32.62	0.69
FCR	1.64	1.51	1.60	1.56	1.61	0.03
Mortality	3.33	6.67	3.33	3.33	3.33	1.31

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

of nutrients and improve secretion of digestive enzymes (Papatisiros *et al.,* 2013; Khan and Igbal., 2016; Dittoe *et al.,* 2018). These benefits of ACAPS may also be dose dependent since its inclusion beyond 0.5% did not elicit similar performance results in broilers at this stage of development, and like the results obtained at the starter phase.

(b) Seven weeks growth performance (4 weeks of starter and 3 weeks of finisher feeding)

The seventh - week (49 day) evaluation was also carried out to determine the benefits of harvesting the birds at that stage of their development. The B4 birds again recorded significantly higher ($p<0.05$) final live weight, bodyweight gain, and daily weight gain than the other groups. The B2 group (0.5% AC) also recorded significantly higher ($p<0.05$) values than the control group although similar to the B3, and B5 group values. The B2, and B4 groups also recorded the best FCR, while the B5 recorded the worst. Again, the average daily feed consumption values were not significant among the groups. These results indicate that 0.5% AC supplementation has beneficial effects on finisher broiler performance and that these effects are potentiated by the addition of aged palm wine to the AC.

(c) Eight-week growth performance (4 weeks of starter and 4 weeks of finisher feeding)

At the end of 8 weeks of feeding, the superior growth performance records of the B4 birds were maintained over the control, B3 and B5 birds. The B2 group however, recorded improved performance indices like those recorded by the B4 group. The control and the B5 groups however recorded the most inferior, and similar indices. Again, no treatment effects were recorded on the feed intake of the birds, indicating that at the various stages of broiler development studied, the birds consumed similar quantities of the experimental diets to achieve different growth performance indices.

Recent studies seem to suggest that AC supplementation has marginal or no impact on growth

Table 4.31: Growth performance of broilers fed AC and ACAPS supplemented diets

(a) 6 weeks

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Initial weight	40.15	40.07	40.00	40.20	39.93	0.09
Final weight	1297.08^{b}	1392.33^{b}	1399.47^{b}	1563.33^{a}	1400.63^{b}	26.19
Weight gain	1256.93^{b}	1352.27^{b}	1359.47^{b}	1523.13^{a}	1360.70^{b}	26.16
ADWG	29.93^{b}	32.20^{b}	32.37^{b}	36.26^{a}	32.39^{b}	0.62
ADFI	60.72	60.89	62.86	65.02	63.62	0.69
FCR	2.03^{a}	1.89^{b}	1.94^{ab}	1.79^{c}	1.96^{ab}	0.02
(b) 7 weeks						
Initial weight	40.15	40.07	40.00	40.20	39.93	0.09
Final weight	1673.59^{c}	1798.42^{b}	1731.80^{bc}	1919.00^{a}	1711.47^{bc}	26.31
Weight gain	1633.43^{c}	1758.35^{b}	1691.80^{bc}	1878.80^{a}	1671.54^{bc}	26.29
ADWG	33.34^{c}	35.89^{b}	34.52^{bc}	38.34^{a}	34.11^{bc}	0.54
ADFI	71.55	71.82	74.17	75.84	75.45	1.10
FCR	2.14^{ab}	2.00^{b}	2.15^{ab}	1.98^{b}	2.21^{a}	0.03
(c) 8 weeks						
Initial weight	40.15	40.07	40.00	40.20	39.93	0.09
Final weight	2050.09^{c}	2204.50^{ab}	2064.14^{bc}	2288.17^{a}	2022.32^{c}	32.61
Weight gain	2009.94^{c}	2164.27^{ab}	2024.13^{bc}	2247.97^{a}	1982.39^{c}	32.59
ADWG	35.89^{c}	38.65^{ab}	36.14^{bc}	40.14^{a}	35.40^{c}	0.58
ADFI	83.19	84.45	85.56	86.99	85.64	1.00
FCR	2.32^{ab}	2.18^{b}	2.37^{ab}	2.17^{b}	2.42^{a}	0.04

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

ADWG = average daily weight gain, DFI = average daily feed intake, FCR = feed conversion ratio of broilers (Hien, Dung, Manh, and Le Minh, 2018; Mongo *et al.,* 2020; Rashidi *et al.,* 2020). For example, Odunsi *et al.* (2007) had observed earlier that higher inclusion levels of charcoal were deleterious to broiler growth. Evans *et al.* (2015) also reported decreased growth performance when higher levels of charcoal were added in broiler diets such that feed energy value was impacted. On the contrary, Dim *et al.* (2018) reported improvements in broiler development during the adult stage even at higher doses of AC in the feed, while Born charcoal and Canarium wood charcoal at 0.4, and 0.2% respectively significantly improved the performance of broilers exposed to aflatoxin contaminated diets (Kana *et al.,* 2014). The variation in performance may be attributed to the biochemical characteristics of the feedstock used in producing the AC (ARDCorp, 2014).

(d) Growth rates of the broilers

Body weight measurements have been routinely used to monitor the nutritional status of broilers expressed as the feed conversion ratio (FCR). While this approach expresses adequately the feed to body weight gain ratio of the birds, it does not reflect the actual growth rate of the birds. According to Flier and Moratos-Flier (2000), and Chimonyo *et al.* (2000), animals with large body frame may have higher body weight, but lower levels of body reserves. In order to understand the actual growth rates of the broiler chicks fed the diets supplemented with AC and ACAPS, we calculated the increase in their initial day-old body weights during the various developmental stages and presented the results in a line graph format in figure VII. The results show that at every stage of development, the birds fed the 0.5% ACAPS (B4) recorded the best growth rate followed by the 0.5% AC diet (B2) group that started showing improvements in growth rate from week six. The control, and the 1.0% AC diet group revealed similar growth rate pattern, while the 1.0% ACAPS group recorded the lowest growth rate, although they tried to improve from the 7th week. The figure also shows that the best growth rates were recorded in all the groups from the 4th to the 6th week of life, while rates seemed to slow down slightly thereafter. These results show that both 0.5% ACAPS and 0.5% AC could be used as growth performance enhancement additives during the finisher stages of broiler production.

The percentage increments in final body weight due to the dietary supplementations of the 0.5% ACAPS and AC during the finisher periods are shown in figure VIII. The results show that the 0.5% ACAPS achieved its best growth promotion effect (20.52%) at the 6th week. The effects however declined steadily over time, such that at 8 weeks, it was 11.61%. The 0.5% AC effect remained stable at about 7.0% throughout the periods considered. Optimal broiler growth promotion effects of the ACAPS would therefore be achieved when supplemented up to 6 and 7 weeks of age.

4.10.4 Economics of producing the finisher broilers fed diets supplemented with AC, and ACAPS.

Table 4.32 showed the economics of producing the finisher broilers fed the AC, and ACAPS supplemented diets for 6, and 8 weeks. The expected revenues at 6 weeks increased progressively up to B4 group before dropping in the group B5 to a value similar to those of the B2, and B3 groups. A similar trend was observed in the profit values such that the B4 group returned the highest revenue, and profit value followed by the other AC, and ACAPS supplemented groups. The control birds returned the lowest revenue and profit margin values.

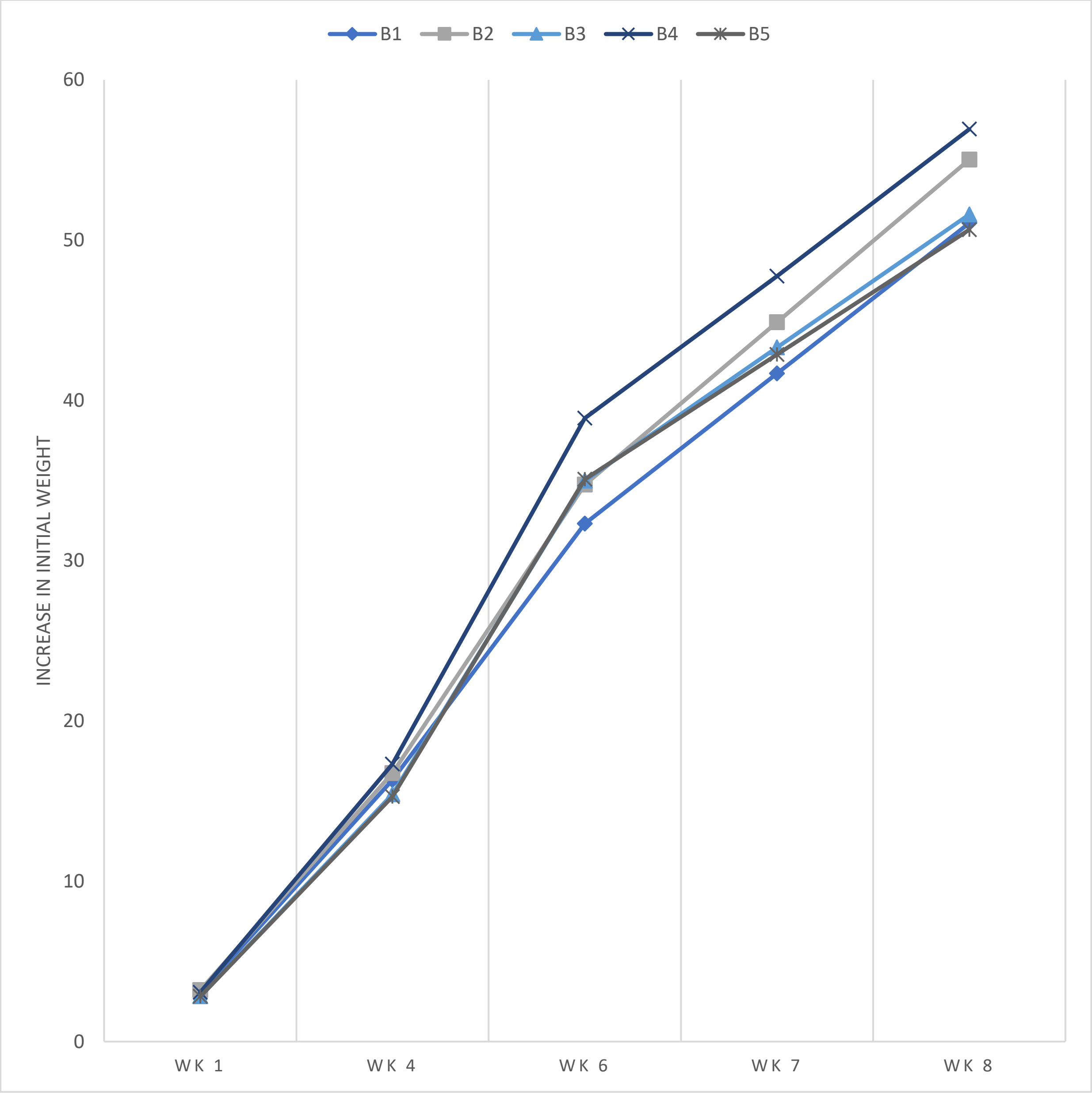

FIG. VII: Increase in the initial body weight of the broiler chicks at different stages

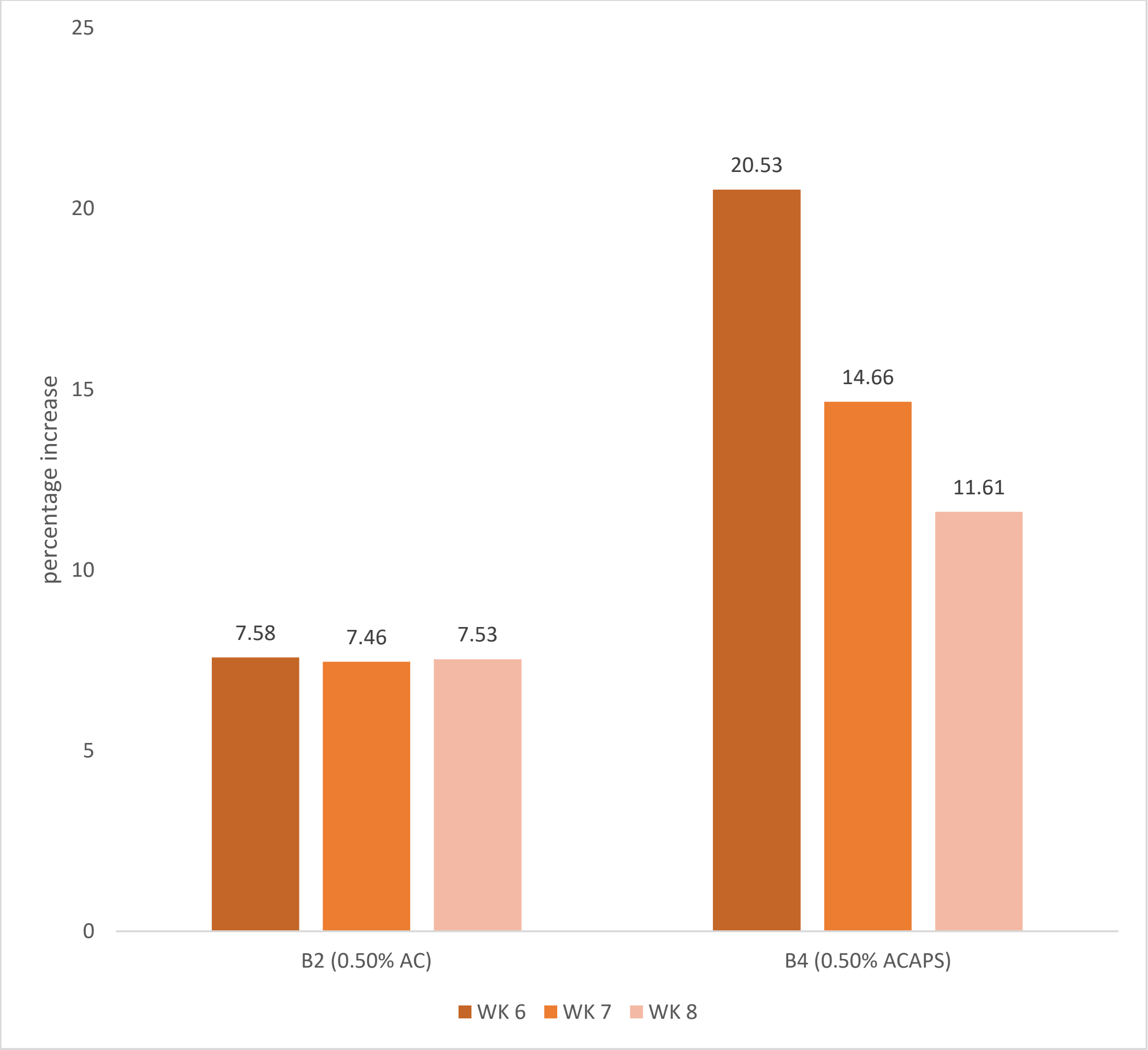

Fig. VIII: Percentage increment in final body weights by the 0.5% AC and ACAPS treated birds

At 8 weeks, the B4 group again, generated the highest revenue, and profit margin values, followed by the B2 group, which improved significantly in its economic returns above the values recorded at 6 weeks. The control, and the remaining AC (B3), and ACAPS (B5) supplemented groups recorded much lower, and similar economic returns. The study therefore shows that the best economic returns at 6, and 8 weeks of production were recorded by the 0.5% ACAPS supplemented group (20.60 and 23.75%, and 11.61 and 13.60% increments in revenue, and profit values respectively at both periods). The 0.5% AC supplemented group recorded the second-best economic returns at 8 weeks, which translated to increments of 7.55, and 9.26% in revenue and profit respectively.

4.11 Carcass, and Organ Characteristics of Finisher Broilers Fed the AC and ACAPS Supplemented Diets

4.11.1 Carcass characteristics of broilers fed the AC, and ACAPS supplemented diets

Carcass characteristics of the birds fed the AC, and ACAPS supplemented diets are shown in Table 4.33. There were no significant differences in all the carcass characteristics values among the AC treated, and the control groups except in their head and shank values. All the groups manifested remarkable dressed weights that ranged from 71.60 - 74.42% which indicates that all the diets supported a proportional cumulative flesh deposition. Birds on the B3, B4, and B5 diets had significantly higher percentage shank weight values ($p<0.05$) than the B2 birds but were similar to the control value. The B3, and B5 birds also recorded significantly higher head weights ($p<0.05$) than the B1, B2, and B4 birds.

The carcass yield results from this study agree with several reports that AC supplementation in broiler diets does not influence carcass yield and cut parts of broiler meat (Majewska *et al.*, 2011, Jiya *et al.*, 2014). The results of the component parts are also within the reported range for broiler meat yield (Ohanaka, 2016; Aviagen, 2019). The carcass yield values are however

Table 4.32: Economics of production of broilers fed AC and ACAPS supplemented diets

(a) 6 weeks

Parameter	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Cost of feed/kg (N)^{a}	144.00	144.00	144.00	144.00	144.00	0.00
Total feed intake (kg)^{b}	2.55^{b}	2.56^{b}	2.64^{ab}	2.73^{a}	2.67^{a}	0.02
Cost of feed consumed (N)^{c}	367.20^{b}	368.16^{b}	380.16^{ab}	393.12^{a}	384.48^{a}	3.11
Feed cost / kg gain(N)	292.32^{a}	272.16^{ab}	279.36^{ab}	257.76^{b}	282.24^{a}	3.95
Final liveweight (kg)^{d}	1.30^{b}	1.39^{b}	1.40^{b}	1.56^{a}	1.40^{b}	0.03
Revenue (N)^{e}	1944.5^{b}	2088.50^{b}	2099.00^{b}	2345.00^{a}	2100.50^{b}	39.31
Profit (N)^{f}	1577.30^{b}	1720.34^{b}	1718.84^{b}	1951.88^{a}	1716.02^{b}	37.09
(b) 8 weeks						
Cost of feed/kg (N)^{a}	144.00	144.00	144.00	144.00	144.00	0.00
Total feed intake (kg)^{b}	4.66	4.73	4.79	4.87	4.79	0.06
Cost of feed consumed (N)^{c}	671.04	680.64	689.76	701.28	690.24	8.04
Feed cost / kg gain (N)	334.08^{ab}	313.92^{b}	341.28^{ab}	312.48^{b}	348.48^{a}	5.07
Final liveweight (kg)^{d}	2.05^{c}	2.20^{ab}	2.06^{bc}	2.29^{a}	2.02^{c}	0.03
Revenue (N)^{e}	3074.50^{c}	3306.50^{ab}	3095.50^{bc}	3431.50^{a}	3033.50^{c}	48.92
Profit (N)^{f}	2403.46^{b}	2625.86^{a}	2405.94^{b}	2730.22^{a}	2343.26^{b}	46.33

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Feed cost /kg = a; Total feed intake =b; Cost of feed consumed (c) = a x b; (d) = final live weight; Revenue (e) = d x cost of broiler meat per kg @ N1500.00 naira; Profit = e – c. feed cost /kg gain = FCR x a

Table 4.33: Carcass characteristics of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Live weight (g)	2150.00	2205.00	2100.00	2253.00	2176.00	27.29
Plucked weight (g)	2025.00	2094.33	1950.33	2117.33	2081.67	27.19
Dressed percentage (%)	71.60	73.34	71.56	74.42	72.95	0.47
Percentage cut	parts %					
Thigh	13.12	12.84	12.43	12.39	12.51	0.26
Drumstick	10.16	11.02	10.70	10.56	10.44	0.14
Breast	19.78	20.78	18.63	20.64	19.85	0.46
Wing	8.12	8.38	8.66	8.35	8.21	0.17
Back	15.74	15.75	16.96	17.68	16.88	0.35
Neck	4.20	3.88	3.90	3.55	4.11	0.13
Shank	4.40^{ab}	3.94^{b}	4.65^{a}	4.69^{a}	4.58^{a}	0.10
Head	2.37^{b}	2.23^{b}	2.66^{a}	2.28^{b}	2.64^{a}	0.05

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

lower than the 76.09 – 76.36% reported by Majewska *et al.* (2011), but higher than the percentage cuts (breast, thigh, and drumstick) values of 15.75, 9.08, and 7.21% reported respectively from broilers fed diets supplemented with 0.3% charcoal for 42 days.

4.11.2 Organ weight characteristics of broilers fed AC, and ACAPS supplemented diets

The organ weight characteristics of broilers fed AC and ACAPS supplemented diets are shown in Table 4.34. Generally, the diets had no effects ($p>0.05$) on the gizzard, heart, lungs, crop, abdominal fat percentages, and intestinal length, but affected percentage liver, and intestinal weights. Birds on the B1, B2, and B5 diets recorded significantly ($p<0.05$) higher percentage liver weights than those on B3 diet but similar to the B4 birds. The B3 birds recorded significantly higher ($p<0.05$) intestinal weight than the B4 birds, while the other groups recorded similar values. Other workers reported decreases in liver weights when plant ash was added to broiler diets (Nwogu *et al.,* 2014; Ohanaka, 2016), which suggests that the feeding of AC to broilers did not cause any hepatotoxicity or excess liver weight. The percentage liver, and gizzard weight values are much higher than the values (1.65, and 0.98% respectively) reported by Majewska *et al.* (2011) in broilers fed diets supplemented with 0.8% charcoal for 42 days.

Lower small intestine weights in broilers have been suggested by researchers to be a sign of improvement in digestive function due to reduced intestinal burden following functional additives feeding in animals (Kim *et al.,* 2006; Sarker *et al.,* 2016a). Other studies reported heavier intestinal tracts in less efficient animals (Van Eerden *et al.,* 2004; de Verdal *et al.,* 2010). The AC did not significantly affect intestinal length although treated birds had longer intestines compared to the control. Longer intestines are often associated with efficient feed digestion, and provision of larger surface area for nutrient absorption (Mabelebele *et al.,* 2014).

Table 4.34: Organ weight characteristics of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Gizzard %	1.72	1.81	1.84	1.98	1.90	0.06
Liver %	2.58^{a}	2.56^{a}	1.94^{b}	2.21^{ab}	2.40^{a}	0.08
Heart %	0.51	0.41	0.48	0.50	0.48	0.02
Lungs %	0.67	0.68	0.62	0.62	0.60	0.03
Crop %	0.39	0.30	0.33	0.33	0.32	0.02
Abdominal fat (%)	0.91	1.13	1.00	0.96	1.07	0.05
Intestinal weight (%)	2.98^{ab}	2.74^{ab}	3.79^{a}	2.51^{b}	2.69^{ab}	0.17
Intestinal length (cm)	208.00	245.67	270.67	248.50	242.00	9.07

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

4.11.3 Skin colour rankings of finisher broilers fed the AC, and ACAPS supplemented diets

Data representing the ranking of skin colour of the finisher broilers fed the diet supplemented with AC, and ACAPS are shown in Table 4.35. Treatment effects were observed in only the breast and thigh skin colours, while there were no effects on the other parts. Specifically, birds on the diet containing 0.5% AC, and ACAPS recorded significantly higher ($p<0.05$) breast, and thigh skin pigmentation or degree of yellowness than the rest of the group. Pigmentation plays a vital role in consumer acceptance, preference, and quality perception of broiler meat products (Liu *et al.,* 2008; Sirri, Petracci, and Meluzzi, 2010; Hamelin and Altemueller, 2012). It is greatly influenced by various dietary feed components, especially carotenoids present in the feed (Hernandes *et al.,* 2005). Yellow maize is a key natural source of carotene present in the experimental feed.

Reports also suggest that the inclusion of gut health-promoting additives such as organic acids in the diet improves the pigmentation of broiler skin through improved pigment, and nutrient absorption in the GIT (Poultry World, 2020). Ponsano, Pinto, Garcia, and Lacava, (2004), reported that the production of oxycarotenoids by gut microorganisms enhances yellowness in the breast, and thigh skin of broiler meat. Therefore, the addition of 0.5% ACAPS in broiler diet may have enhanced pigment, and nutrient absorption which led to increased deposition of pigment on the breast, and thigh skin leading to their increased yellowness compared to other groups.

Table 4.35: Skin colour ranking of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Shank colour	101.44	101.67	101.00	101.33	101.67	0.16
Breast skin colour	101.00^{b}	101.00^{b}	101.00^{b}	102.33^{a}	101.33^{b}	0.29
Thigh skin colour	101.00^{b}	101.00^{b}	101.33^{b}	102.67^{a}	101.33^{b}	0.19
Back skin colour	101.00	101.33	101.00	102.00	101.00	0.15
Abdominal fat colour	101.00	101.67	101.00	101.00	101.33	0.11
Vent colour	101.00	101.33	101.33	101.67	101.67	0.16

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

4.12 Faecal Characteristics, and Environmental Effects of Feeding AC and ACAPS Supplemented Diets

4.12.1 Concentrations of faecal minerals

The data on mineral content of the faeces voided by the experimental broiler birds are shown in table 4.36. Generally, the order of abundance of the minerals in the faecal materials was Ca> K> P> Na> Mg> Fe> Mn> Zn> Cu > Cl. This mimics the order of mineral concentrations in both the starter, and finisher diets. The result highlighted higher levels of the minerals in the faeces, than the diets. For example, the levels of all the minerals in the control birds' faeces were much higher than that of the control diets with Ca, K, and Mn concentrations more than tripling (Table 4.25).

The concentrations of P, Mg, K, Fe, Cu, Zn, Mn, and Cl in the faeces were higher in the AC and ACAPS treated groups, and increased progressively across the diets, such that the B5 group recorded significantly higher ($p<0.05$) concentration values than the other treated groups, and the control values. All other AC treated diets also recorded significantly higher levels for these minerals than the control. The faecal concentrations of Ca, Na, and Cl however reduced progressively with increase in dietary supplementation of AC and ACAPS in the diets. The B5 therefore recorded the least faecal concentrations, while the control group had more Ca, Na and Cl in their excreta. These results mimic the dietary mineral concentrations in which the control diets recorded highest Ca, and Na concentrations, and the lowest concentrations of the other minerals. Therefore, the reduction in faecal concentrations could be due to the need of the birds to make up for the reductions in their diets because of AC, and ACAPS supplementations. Ohanaka (2016) reported similar result in broilers fed palm kernel shell ash (PKSA) as a mineral supplement in finisher broiler rations in which faecal Ca, Na, Mn, Fe, and P concentrations reduced due to PKSA inclusion in the diets.

Table 4.36: Faecal mineral concentrations of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Calcium (mg/kg)	35113.28^{a}	35091.45^{b}	34970.98^{c}	34881.07^{d}	34759.10^{e}	35.35
Magnesium (mg/kg)	2983.47^{e}	3061.50^{d}	3199.78^{c}	3283.13^{b}	3437.18^{a}	42.93
Potassium (mg/kg)	14166.30^{e}	14430.42^{d}	14924.35^{c}	15320.82^{b}	16312.22^{a}	205.42
Phosphorus (mg/kg)	5180.47^{e}	5710.61^{d}	6212.80^{c}	6713.67^{b}	7061.59^{a}	180.77
Sodium (mg/kg)	3939.11^{a}	3604.44^{b}	3209.15^{c}	3105.05^{d}	3005.73^{e}	93.30
Manganese (mg/kg)	90.23^{e}	107.98^{d}	128.93^{c}	135.72^{b}	143.18^{a}	5.20
Iron (mg/kg)	376.96^{e}	449.74^{d}	612.37^{c}	712.88^{b}	971.61^{a}	56.11
Copper (mg/kg)	12.38^{e}	14.02^{d}	16.23^{c}	17.16^{b}	19.02^{a}	0.63
Zinc (mg/kg)	45.16^{e}	50.81^{d}	58.93^{c}	60.80^{b}	67.88^{a}	2.12
Chlorine (mg/kg)	7.29^{e}	7.85^{d}	9.70^{c}	8.80^{b}	10.44^{a}	0.31

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

4.12.2 Environmental effects of feeding the AC and ACAPS supplemented diets to broilers

The results on the effects of AC and ACAPS supplementation on faecal and litter eco-characteristics are shown in table 4.37. The faecal moisture content was low enough to support fungal growths which could be harmful to the birds. The faeces were also high in crude protein, such that B3 – B5 samples recorded significantly higher ($p<0.05$) protein values than the control, and B2. The nitrogen values expectedly followed the same trend. The higher values probably reflect the ability of the AC to adsorb dietary nutrients such as water, proteins, fats, and vitamins, which are then voided with the faeces (Wang *et al.*, 2006). The crude protein and nitrogen values were however lower than the values recorded in laying birds (Table 4.17). Odour and fly infestation results are however similar to those recorded in the layer study and support the findings of Durunna *et al.* (2018), that both air quality and fly population could be controlled by feeding broilers with diets supplemented with wood charcoal.

4.13 Haematological and Serum Biochemical Characteristics of Broilers fed AC and ACAPS Supplemented Diets

4.13.1 Haematological characteristics of broilers fed AC and ACAPS supplemented diets

The haematological results in the table 4.38 show that the inclusion of AC, and ACAPS in the diets of the broilers generally, resulted in significant improvements in all the parameters measured ($p<0.05$) beyond that of the control, except the clotting time results which followed a reverse trend.

Blood parameters improved progressively with charcoal addition. This contrasts from the blood results in the layer study, which showed decreasing haematological, and serum biochemical values with increasing dietary inclusion of AC and ACAPS in the diets. This

Table 4.37: Effects of AC and ACAPS supplementations on faecal and litter Eco-characteristics

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Faecal moisture	8.24^{b}	8.24^{b}	7.74^{c}	8.26^{b}	8.50^{a}	0.07
Faecal crude protein (%)	19.44^{b}	18.55^{c}	20.02^{a}	20.09^{a}	19.68^{ab}	0.16
Odour	High	Low	Low	Low	Low	-
Fly infestation	+	-	-	-	-	-
Nitrogen (%)	3.11^{a}	3.01^{b}	3.20^{a}	3.19^{a}	3.14^{a}	0.02

indicates that these additives supported better blood formation possibly due to improved bioavailability of iron, and copper from the diets. It is therefore probable that the ionic forms of Fe, and Cu in AC are readily bioavailable to the birds through improved uptake from the digestive tracts (Ohanaka *et al.,* 2018b). Dim *et al.* (2018) also reported improvements in haematological parameters of broilers fed corn stover biochar even at 4 - 6% dietary inclusion, while no effects were observed by Majewska *et al.* (2009), and Kana *et al.* (2014) with biochar feeding in broiler chicken.

The RBC values in this study were however higher than those reported for Arbor Acre broilers (2.59 x 10^6 µl) (Talebi, Asri–Rezaei, Rozeh–Chai, and Sahraei, 2005) but within the normal range for chicken. All the other blood values obtained in this study agree with normal reference range for chicken (Aiello and Mays, 1998; Talebi *et al.*, 2005; Al Nedawi, 2018).

The significant decrease in blood coagulation time (BCT) with increasing levels of AC, and ACAPS inclusion in the diets was also observed in the layer study and is within the range for avian specie (2 to 10 minutes) as reported by Obidimma (2009). This result suggests that AC contains minerals in the forms that reduce BCT, such as calcium which has been reported to enhance blood clotting ability (Waldroup, 1997).

4.13.2 WBC and differential counts in broilers fed AC and ACAPS supplemented diets

Table 4.39 represents the results of the white blood cell (WBC) count, and its differential values across the various AC, and ACAPS treatments. The WBC, and lymphocyte values differed significantly ($p<0.05$) among the treatments, while the heterophil, eosinophil, monocyte, and basophil counts remained unaffected ($p>0.05$). The WBC values increased with increasing inclusion levels of the AC, and ACAPS in the diets, with the treated birds recording significantly higher values ($p<0.05$) than the control birds. The ACAPS treated birds also recorded higher WBC values than AC treated birds. The high values of the WBC counts

Table 4.38: Haematological indices of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Hb (g/dl)	13.10^{c}	14.00^{b}	14.30^{b}	14.27^{b}	14.80^{a}	0.15
PCV (%)	33.93^{d}	42.00^{c}	42.77^{b}	43.00^{b}	44.40^{a}	0.45
RBC (X10^{6} µl)	7.10^{d}	7.50^{c}	8.00^{b}	8.20^{a}	8.30^{a}	0.12
MCV (fl)	128.00 ^{b}	129.60 ^{b}	134.40 ^{a}	136.00 ^{a}	136.00 ^{a}	0.96
MCH (ƿg)	25.43 ^{d}	26.56 ^{c}	27.68 ^{b}	28.00 ^{b}	28.80 ^{a}	0.33
MCHC (ƿg)	30.00 ^{e}	32.10 ^{d}	33.00 ^{c}	34.00 ^{b}	34.20 ^{a}	0.41
BCT (sec)	41.00 ^{a}	38.00 ^{b}	36.00 ^{c}	33.00 ^{d}	30.00 ^{e}	1.05

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

observed in the birds fed the ACAPS and AC supplemented diets indicate that these groups had superior immune status, and better ability to fight disease compared with control birds (Robert, Murray, Daryl, Grammer, and Rodwell, 2003). The values obtained in this study are however within the normal range for chicken (Mitruka and Rawnsley, 1997). The lymphocyte value was also significantly higher in the B5 birds than the control birds ($p<0.05$) but was similar to the other AC treated birds ($p>0.05$). Increases in lymphocyte values of the blood indicates enhanced immunological well-being of the body (Guyton and Hall, 2006).

No significant difference was observed across the treatment values for the heterophil, eosinophil, and monocytes ($p> 0.05$), while basophil were not observed. This suggests that there was no allergic reaction because of AC supplementation (Robert *et al.*, 2003). All the differential counts obtained in this study were also within normal range. These improvements in haematological values observed in the AC supplemented groups conform to the findings of Nwogu (2013), and Ohanaka (2016), in pullets, and broilers fed diets supplemented with plantain ash, and palm kernel shell ash respectively.

4.13.3 Serum nutrient compositions in broilers fed AC and ACAPS supplemented diets

Table 4.40 shows the results of serum nutrient measurements. Generally, B5 birds recorded the highest total serum protein, albumin, globulin, glucose, and cholesterol values. The B3 – B5 values of the proteins were significantly higher ($p<0.05$) than the control values. The urea value increased progressively with increasing AC, and ACAPS supplementation levels, such that the values from the treated birds were significantly higher ($p<0.05$) than the control value. Improvement in the total serum protein (TSP) value with increasing levels of AC supplementation could be due to increased synthesis of protein caused by improved liver functions, increased protein absorption from the small intestine and decreased losses from the renal system, all indicating improvements in protein handling by the birds (Zantop, 1997).

Table 4.39: WBC differentials of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
WBC (X10^3 µl)	5.00^d	5.50^c	5.50^c	5.70^b	6.00^a	0.09
Lymphocyte (%)	24.67^b	25.00^{ab}	25.67^{ab}	25.00^{ab}	26.00^a	0.18
Heterophils (%)	66.00	65.00	66.30	66.00	66.00	0.26
Eosinophil (%)	3.00	3.00	1.33	2.00	2.00	0.30
Monocyte (%)	6.33	7.00	6.67	7.00	6.00	0.32
Basophil (%)		-	-	-	-	-

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

Table 4.40: Serum biochemical indices of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Total serum protein (g/dl)	8.15^{b}	8.25^{b}	8.52^{a}	8.60^{a}	8.60^{a}	0.05
Albumin (g/dl)	4.37^{b}	4.40^{b}	4.50^{a}	4.50^{a}	4.54^{a}	0.02
Globulin (g/dl)	3.78^{b}	3.92^{ab}	4.02^{a}	4.10^{a}	4.06^{a}	0.04
Albumin/Globulin ratio	1.15	1.12	1.12	1.10	1.12	0.01
Urea (mg/dl)	25.60^{a}	24.60^{b}	24.10^{c}	24.00^{c}	23.33^{d}	0.20
Glucose (mg/dl)	156.40^{d}	158.00^{c}	165.00^{b}	165.20^{b}	166.00^{a}	1.09
Cholesterol (mg/dl)	128.00^{b}	128.30^{b}	130.00^{ab}	131.00^{ab}	132.00^{a}	0.57
Creatinine (pg/dl)	1.40	1.40	1.30	1.30	1.25	0.03

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

All the protein values were significantly higher ($p<0.05$) than the values reported in broilers fed palm kernel shell ash (Ohanaka, 2016), and pullets fed plantain ash (Nwogu, 2013). It is therefore probable that AC supplementation may enhance protein metabolism functions in the liver because of better bioavailability of its mineral contents (Nwogu, 2013). The urea values were however lower than the range of 24.57 – 41.20 mg/dl reported in pullets by Nwogu (2013), but similar to the 19.03 – 24.20 mg/dl reported in broilers by Ohanaka (2016). These results imply that the protein levels in the diets are sufficient to sustain or support the normal protein levels in the blood. Total serum protein, and albumin are associated with dietary protein availability, and will decline due to dietary protein deficiency, while increases in globulin suggest improved immune response. The total proteins, albumin, and globulin values obtained in this study were however within the reported normal range for chicken (Aiello and Mays, 1998).

The creatinine values were not affected by the AC and ACAPS supplementation. Serum urea, TSP, and creatinine have been associated with both the quality, and quantity of proteins in the diet (Iyayi and Tewe, 1998). Higher concentration of serum urea nitrogen observed in the birds fed the control diet signifies impaired synthesis, and lower utilization of proteins (Denli, Blandon, Guyno, Salado, and Perez, 2009). The glucose values obtained across the means were again within the literature limits reported by Mitruka and Rawnsley (1997). Normal glucose level in birds indicates adequate synthesis of glucose from its major precursor (propionate) in the liver (Houtert, 1993).

Contrary to the findings of this study, Jiya *et al.* (2014), and Dim *et al.* (2018) reported decreased serum cholesterol in broilers as the dietary activated charcoal level was increased. Supplementing AC in the layer diets also decreased serum cholesterol in this study. Variations in serum cholesterol content have been linked to the differences in the breed of chicken, nutritional pattern, type of feed, environmental factors, and the test ingredients used (Nworgu *et al*., 2007). The range of values recorded in this study is however within the reported normal reference range (100 – 150 mg/dl) for chicken (Sturkie, Hazel, and Wood, 2000). Therefore, AC supplementation consistently maintained the serum cholesterol of the birds in all the diets indicating that AC, and ACAPS may enhance intestinal cholesterol absorption or synthesis (Aiello and Mays, 1998).

Generally, the feeding of AC to broilers seems to exert its major effects in the GIT where it causes better uptake of essential minerals, improved digestion, and absorption of proteins, lipids, and carbohydrates in birds. These effects are reflected by the higher serum nutrients observed in these groups of birds.

4.13.4 Serum enzymes activities in the broilers fed AC and ACAPS supplemented diets

Table 4.41 represents the serum enzymes values of the broilers fed the AC, and ACAPS supplemented diets. Aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) were significantly lowered below the control bird values ($p<0.05$) with increased AC inclusion in the diets. The serum enzymes are usually produced in the liver, and other organs, and their clinical assay constitute the so-called liver function tests.

The AST, ALT and ALP are enzymes contained mostly in liver cells which enter the blood only when the cells are damaged or destroyed. The presence in the blood of significant quantities of these liver enzymes indicates increased liver tissues damage or injury. The higher AST values recorded in the birds fed the control diet indicates liver lesions because of their lower feed ingredients utilization, and or slow growth rate. The increased level of AST might not be a signal for worries as the thresh-hold was not exceeded. When AST activity is however greater than 800 IU/L, it is indicative of a severe hepatic disorder (Melillo, 2013), and may possibly be due to increased metabolic activities in the liver (Szabo *et al*., 2005). The AST, and ALP values from this study were much lower than the values reported by Nwogu (2013)

Table 4.41: Serum enzyme activities of broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
ALP (IU/L)	67.00 ^{a}	66.00 ^{ab}	65.00 ^{abc}	64.00 ^{bc}	63.50 ^{c}	0.44
ALT (IU/L)	12.20 ^{a}	12.00 ^{a}	10.80 ^{b}	10.30 ^{c}	10.00 ^{c}	0.24
AST (IU/L)	9.00 ^{a}	8.70 ^{b}	8.00 ^{c}	8.00 ^{c}	8.00 ^{c}	0.12

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

in pullets fed plantain ash, while the ALT values were higher. Ohanaka (2016) also reported lower ALT, and ALP values but similar AST values in broilers fed palm kernel shell ash supplemented diets. Serum ALT activities are usually low in chicken tissue, but may increase due to tissue damage (Zantop, 1997). However, diagnostic value of serum ALT in birds is usually poor since birds with severe liver damage may have normal ALT activities. The ALT values reported in this study are within the normal range reported for chicken (Campbell and Cole, 1986).

Contrary to the result in the layer experiment, there was again, decreased ALP activity in the AC treated broilers compared to the control. Increased serum ALP has been linked with increased osteoblast induced bone remodelling (Attia *et al*., 2000; Harr, 2002), and improved blood ionic Ca. It is therefore possible that the decreased ALP activity in the ACAPS treated birds is because of reduced bone remodelling in the broilers.

4.13.5 Serum mineral compositions of broilers fed AC and ACAPS supplemented diets

The serum electrolytes concentrations of the broilers fed diets containing the AC, and ACAPS are shown in table 4.42. The Ca, P, and K levels increased significantly with increasing AC, and ACAPS supplementation in the diets. The levels in AC treated birds were significantly higher than those of the control ($p<0.05$), while Na concentrations decreased in ACAPS treated birds compared to the other groups. There was however no significant treatment effect on the chloride, and bicarbonate values. Serum electrolytes help to maintain the cellular integrity, pH and regulation of neuro-muscular functions (Cheesbrough, 2000). Elements such as sodium, and potassium are particularly known to regulate acid–base balance and nerve impulses. The significant differences in serum calcium obtained in this study may be attributed to better calcium absorption from AC supplemented diets (Duwa, Oyawole, and Njidda, 2012), despite its dilution/lower content in the broiler diets (Table 4.25). The significantly higher serum Ca

Table 4.42: Serum mineral compositions in broilers fed AC and ACAPS supplemented diets

Parameters	B1 Control	B2 0.50% AC	B3 1.00% AC	B4 0.50% ACAPS	B5 1.00% ACAPS	SEM
Calcium (mg/dl)	10.40^{c}	10.50^{c}	11.00^{b}	11.30^{a}	11.40^{a}	0.11
Phosphorus (mg/dl)	3.17^{c}	3.30^{c}	3.60^{b}	3.97^{a}	4.00^{a}	0.09
Sodium (mmol/l)	140.00^{a}	140.20^{a}	139.00^{a}	136.00^{b}	136.00^{b}	0.57
Potassium (mmol/l)	2.70^{d}	2.93^{c}	3.00^{c}	3.20^{b}	3.40^{a}	0.07
Chloride (mmol/l)	99.00	100.00	100.00	102.00	98.00	0.35
Bicarbonate (mmol/l)	25.00	26.00	26.00	28.00	27.00	0.27

Means with different superscript on the same horizontal row are significantly different @ $p<0.05$

and P recorded in ACAPS group compared to the control or the AC groups may be an effect of the organic acid which has a lowering effect on gut pH values, thus increasing their absorption from the gut into the bloodstream. Kishi *et al.* (1999) reported improved Ca solubility and absorption in rats fed acetic acid in their diet. Okoli *et al.* (2014) reported that plant ash as calcium source influences better absorption of calcium in the birds. Ohanaka (2016) reported higher P, and K, but lower Ca values in broilers fed palm kernel shell ash supplemented diets, while the other serum mineral values were similar. The serum Na values obtained were within the normal ranges of 130 – 150 mmol/l as reported by Mitruka and Rawnsley (1997) for broilers.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

This study showed that the carbonization of the blend of pig dung, palm kernel shell and bamboo could yield a slightly alkaline activated charcoal with a high carbon content of 75.35% and total ash content of 13.13% which contained P, K, Ca, and Mg as its major macro-mineral constituents.

The inclusion of activated charcoal (AC) in the experimental layer, and broiler diets made the diets less bulky, and more water absorbent, and increased the binding of nutrients such as crude protein, ether extract, crude fibre, and total ash in the AC-treated diets. Generally, AC supplementation in the experimental layer diet resulted in significant reductions in the dietary Mg, K, P, Mn, Fe, Cu, and Zn levels, while increasing the levels of Ca, Na, and Cl beyond the control values. In contrast, AC supplementation in the broiler diets resulted in a progressive reduction in the Ca, Na, and Cl levels, while K, P, Fe, Mn, Cu, and Zn levels were increased. AC supplementation also increased the mineral ratios, and diet electrolyte balance (dEB) of the layer diets, while they were decreased in the broiler diets.

The laying performance was enhanced by the AC and activated charcoal – aged palm sap blend (ACAPS) supplementation in the layer diets, with the birds fed the 1.00% ACAPS diet laying 12.84% more eggs than the control and recorded less weight loss due to egg laying intensity. The ACAPS diets also improved most of the yolk, and ovarian parameters, but reduced most haematological and serum biochemical constituents below control values. Contrarily, the dietary AC, and ACAPS supplementation improved all the haematological parameters and the serum alkaline phosphate (ALP) and bicarbonate levels.

The dietary supplementation of 0.50% AC, and ACAPS improved the final liveweights, weight gains, and feed intake of broilers more than the control, and the 1.00% AC, and ACAPS diets, both at the 7th day, and the 28th day of feeding. At the broiler finisher levels, the birds fed the 0.50% ACAPS supplemented diets outperformed the other groups in terms of final weight, and weight gains up to the 49th day of feeding, while by the 56th day, the 0.50% AC group have levelled up. These growth performance results also support significant improvements in the revenue and profit margins accruing from the sales of the birds from these groups after six, and eight weeks of feeding.

The AC, and ACAPS supplementation caused improved uptake of K, P, Mg, Fe, Mn, Cu, and Zn by the laying hen, and Ca, Na, and Cl by the broilers as shown by the lower than their control values in the faeces of the treated birds. The AC dietary supplementation could also be used to improve the air quality in the poultry pen due to its ability to reduce odour, and flies' infestation of the litter.

5.2 Recommendations

The supplementation of activated charcoal (AC) and activated charcoal - aged palm sap (ACAPS) at 1.00% levels in the layer diets are recommended based on their positive effects on laying performance, the reproductive tract, and reductions in the mineral content of the faeces.

The effects of AC and ACAPS supplementation as feed additives in the broiler diets is dose-dependent, and therefore should not exceed 0.5% inclusion beyond which it becomes less tolerable and impacts on broiler performance. The, 0.5% ACAPS however, proved to be the most beneficial based on the superior growth performance indices, and the higher profit margins it supported.

The inclusion of up to 0.50% of activated charcoal products in poultry diets is also recommended as a solution to the odour, and nuisance fly problems associated with poultry farming.

The supplementation of ACAPS at 0.50 and 1.00% in broilers and layer diets respectively is therefore recommended as feed grade additives for optimal performance in poultry production.

Further studies can be conducted to understudy the influence of agro-waste derived AC and ACAPS supplementation on intestinal microbial ecology and gene expression in poultry.

5.3 Contribution to knowledge

Agro–wastes are of limited economic value to the farmers and often, constitute environmental hazards when not properly managed. To create additional value for agro-waste resources outside its original use as manure and fuel, the present study was able to achieve the following.

1. The development of a simple pyrolytic technology that could readily convert a blend of agro by-products like pig dung, palm kernel shell and bamboo, into an activated charcoal (AC) product that can be of economic benefit to the farmer.
2. The production of AC and activated charcoal-aged palm sap (ACAPS) products as feed-grade additives for use in poultry.
3. The study established that the optimal supplementation levels of AC and ACAPS additives in the diets of laying hen improved laying performance and reproductive tract development in treated hen at 1.00% AC and ACAPS inclusions respectively.
4. The establishment of the optimal levels of AC and ACAPS additive supplementation in broiler diets to be dose dependent at 0.50% or 0.5 kg per 100 kg of feed respectively.
5. Supplementing ACAPS in the diets of laying hens enhanced egg laying performance, producing 12.84% more eggs which translates to extra 12 eggs for every 100 eggs laid, while reducing weight loss due to egg laying intensity.
6. The supplementation of AC and ACAPS improved broiler performance by 154.41 and 238.08 g or 7.53% and 11.61% increase in final body weight at 56 days of feeding.

However, the growth promoting effect of 0.50% ACAPS was best at 42 days of feeding yielding 266.25 g or 20.52% increment in final body weight.

7. The supplementation of AC and ACAPS in the experimental diets led to the production of eco-friendly poultry faeces with reduced odour emission and fly infestation.

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