The Journal of Animal & Plant Sciences, 30(3): 2020, Page: 517-526
ISSN (print): 1018-7081; ISSN (online): 2309-8694

Review Paper

ENZYMATIC TREATMENT OF PALM KERNEL CAKE IMPROVES INTESTINAL HEALTH, GUT MICROBIOTA AND IMMUNE SYSTEM OF BROILERS.

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ABSTRACT

Palm kernel cake is a very important agro-industrial by-product from the production of palm kernel oil. It contains a lot of nutrients needed by chicken for growth and productivity but this potential is hindered because of the presence of mannan and other anti-nutritional factors. Mannans are made up of simple sugar D-mannose. Mannans comprise of a linear backbone of mannose residues joined by β-1, 4-mannosidic linkages comprising glucomannan, galactomannan and galactoglucomannan depending on carbohydrates or acid substitutions in the backbone. Enzymes have the ability to degrade this mannan component in order to make the nutrients available to the animals. Mannanoligosaccharides improve the development of small intestine of chicks during pre- and post-hatch periods, increasing the villi height, crypt depth and number of goblet cells per villus on the day of hatch. The activities of these enzymes help in increasing the population of beneficial microorganisms residing in the gastrointestinal tract of chicken. The surge in the beneficial microorganisms residing in the gastrointestinal region of chicken helps in boosting their immune system. Feeding palm kernel cake promotes growth and prevents diseases by boosting the immune system of broilers.

Key words: Gut microbiota; immune system; enzyme; intestinal morphology; palm kernel cake.

https://doi.org/10.36899/JAPS.2020.3.0061 *Published online March 25, 2020*

INTRODUCTION

There is and continuous to be tremendous growth in the oil palm industry in the world today. The result of these, are the production of lots of agro-waste by-products associated with the industry. Notable by-products are the chaffs left after the extraction of palm oil, the shell and palm kernel cake (PKC) from the production of palm kernel oil (Akpan *et al*., 2017). These agro-industrial by-products are very important in so many spheres of life. However their use as animal feed especially chicken is limited due to their nutritional characteristic and availability (Alimon, 2004; Gonzaga Neto *et al*., 2015; Oliveira *et al*., 2015; Sundu *et al*., 2005). The oil palm is a perennial tree which thrives well in the tropics and sub-tropic regions of the world (Africa, Asia and South America) where most of the countries are classified as developing. Many households in the developing countries depend on it for food (Ofori-Boateng and Lee, 2013). The demand for poultry and its product keep rising because it serves as an easy avenue of animal protein. The increase in demand for poultry and their product globally has resulted in the increase of prices of feed for poultry. Several researches have shown that palm kernel cake is widely used to feed ruminants compared to poultry (Alimon 2004; Sundu *et al.,* 2005). This low usage has been attributed to the presence of anti-nutritional factors and certain toxins present in them. The developing countries that produce oil palm in large quantities are not able to utilize the palm kernel cake because the farmers are not able to buy and rear ruminants mostly cattle which has the ability to digest the non-starch polysaccharides. The palm kernel cake produced are wasted or to a limited extent used as a source of fuel. Most of these farmers keep chicken on subsistence basis because of the high cost of feeding them on commercial scale. Various researches have shown that palm kernel cake contains a lot of nutrients that can be utilized by poultry but because of the presence of non-starch polysaccharides (NSPs) such as mannan and xylan, together with anti-nutritional factors its utilization is low. Mannan, structurally makes up about 35% of the cell wall of PKC/PKM but cannot be degraded unless it is acted upon by β-mannanase, which degrades it into shorter-chains (van Zyla *et al.,* 2010). The ability of most birds to make use of PKC is lacking because of their inability to produce β-mannanase.

There have been varying reports about the nutrient composition of PKC with the method of oil extraction being the determinant. There are two main methods of oil extraction; the solvent method and the mechanical/expeller method with the mechanical method yielding more ether extract. Aladi *et al*. (2013) reported that solid state fermentation could be used as a technical way of increasing the nutrient composition or nutritive value of agro-industrial by-products. A group of simple sugars (D-mannose) make up mannans. Mannans consists basically of residues of mannose being linked by β-1, 4-

mannosidic linkages comprising glucomannan, galactomannan and galactoglucomannan. This link depends on the substitution of acids or carbohydrate in the framework (Olaniyi *et al.,* 2013). Among the various methods of degradation, the one by enzymes is the one often selected as a result of the ease of implementation. Four key enzymes work sequentially in the breakdown of mannans; endo(1 →4)-β-mannanase, exo(1 → 4)-β-mannanase or β-mannosidase, β-glucosidase and α-galactosidase. These enzymes help in assisting the gut microbiota in their metabolism activities by producing simple sugar components that serve as the products from the breakdown of mannans (Malgas *et al.,* 2015). This review discusses the importance of enzymes in increasing the nutrient composition of PKC and how it boosts the immune system and microbiota composition of chicken.

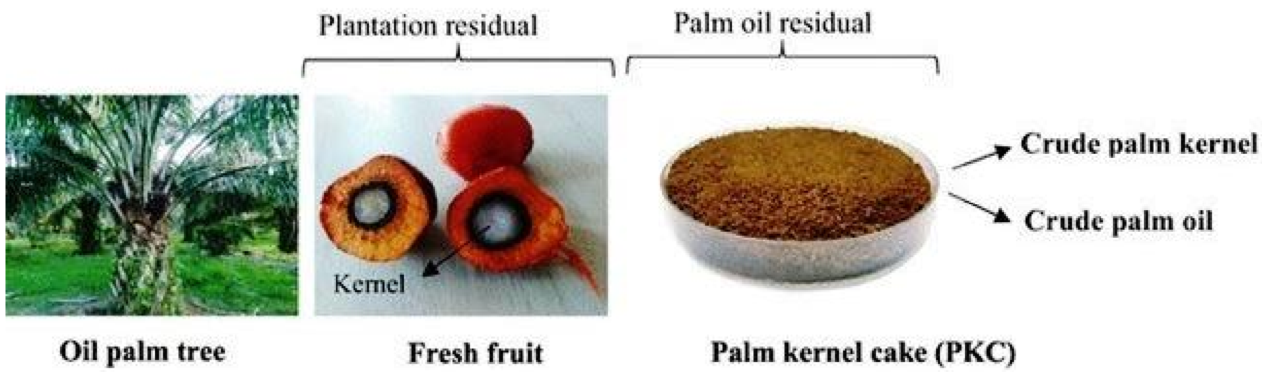

Fig. 1. Oil palm tree, palm fruit and palm kernel cake (Bashirat *et al.*, 2018)

Composition of PKC: Using PKC as feed for ruminants is relatively common in most countries with ration for cattle having an inclusion level of 50%. Due to climatic conditions and geographical locations, composition of PKC may differ from one area to another. Of the two methods of oil extraction, (expeller/mechanical and solvent extraction), the mechanical method produces higher level of ether extract than those produced by solvent extraction. Depending on the oil extraction method, PKC can contain 4-5%, 0.70–0.90%, and 10–17% of ash, ether extract, and crude fiber respectively (Chong *et al.,* 2008). In a study by Almaguer *et al.,* (2014), the neutral detergent fiber (NDF) component of palm kernel cake was found to be 779g/kg whiles the acid detergent fiber was 494g/kg. The NSP and lignin content was found to be 466 and 136 g/kg respectively (Knudsen 1997) and Abdollahi *et al.* (2015) discovered that about 96% are insoluble. According to Mohd-Jafar and Jarvis, (1992), PKC consist of 580g/Kg mannan, 120g/Kg cellulose and 40g/Kg xylan. Zhang *et al.,* (2009), extracted up to 488g/Kg D-mannose from PKC. Ramachandran *et al.,* (2007) also reported that the composition of nutrients are18.6%, 37% and 4.5% of crude protein, dietary fiber and ash respectively.

Biochemical studies reported of high quantity of mannan making up the carbohydrate component of palm kernel cake. Therefore, it had been estimated that mannan constituted 57.8% of hemicellulose content of PKC composition (Ong *et al*., 2004; Cerveró *et al.,* 2010; Azman *et al.,* 2016). PKC also contains 4.71% glucose and galactose, 3.78% xylose (Jahromi *et al.,* 2016).The fatty acid content present in oil or fat determines the nutrient content. Among the two types of fatty acids, the unsaturated ones are more nutritionally valuable than saturated ones (Murano, 2003). There has been increased focus towards using microbes to upgrade low quality lignocelluloses and other non-digestible plant parts into feed for ruminants and other farm animals. Because of the ability of solid-state fermentation process to create a natural environment condition for micro-organisms like fungi, it is mostly used to enrich the surplus of agricultural produce. Under this condition, growth is preferred due to the fact that it makes use of low technology and the problem of waste disposal is curtailed as all the products will be made used of in animal feed. Fermentation which is simply defined as a metabolic process that breaks down sugars in the absence of oxygen has been found to be useful in increasing the nutrient composition of PKC and eventually aid in digestion. The unfermented ones have more dry matter content, high ether extract, high NFE, but lower CP level than the fermented mixture. Aladi *et al.,* (2013) also confirmed the high levels of non-starch polysaccharides in the unfermented mixture of PKC and attributed it to increase in inclination towards water molecules by the new complexes and molecules formed. Water molecules play a very important role in ensuring that the shape and structure of some polymers like proteins, nucleotides and carbohydrates are stabilized (Panigrahi and Powell, 1991; Aladi 2016).

The various activities of microbes in the fermentation process helps in decreasing the level of ether extract and NFE fractions because they are used as carbon source which result in increasing the CP level and also help to increase the microbial mass turnover. Iyayi and Bina (2005) reported that during fermentation, microbes produce enzymes which are able to degrade cellulose causing a reduction in the fiber content. The investigations by Aladi (2013) also showed that aside PKC having as high as 43% mannose in the form of polymer, it increases the population of non-pathogenic microbes and promotes the synthesis of immunoglobulins. Ezieshi and Olomu (2007) reported that crude protein content ranges from (14.5–19.24%). PKC after chemical analysis consisted of the following components; cellulose, hemicellulose, lignin, proteins, oligosaccharides, phenolics and phytosterols. It also has functional groups which perform therapeutic activities and many antioxidants (Fan *et al.,* 2014). In the formulation of diets for broilers, together with methionine and lysine, it could be added up to 40% without adverse effect (Sundu *et al.,* 2006).

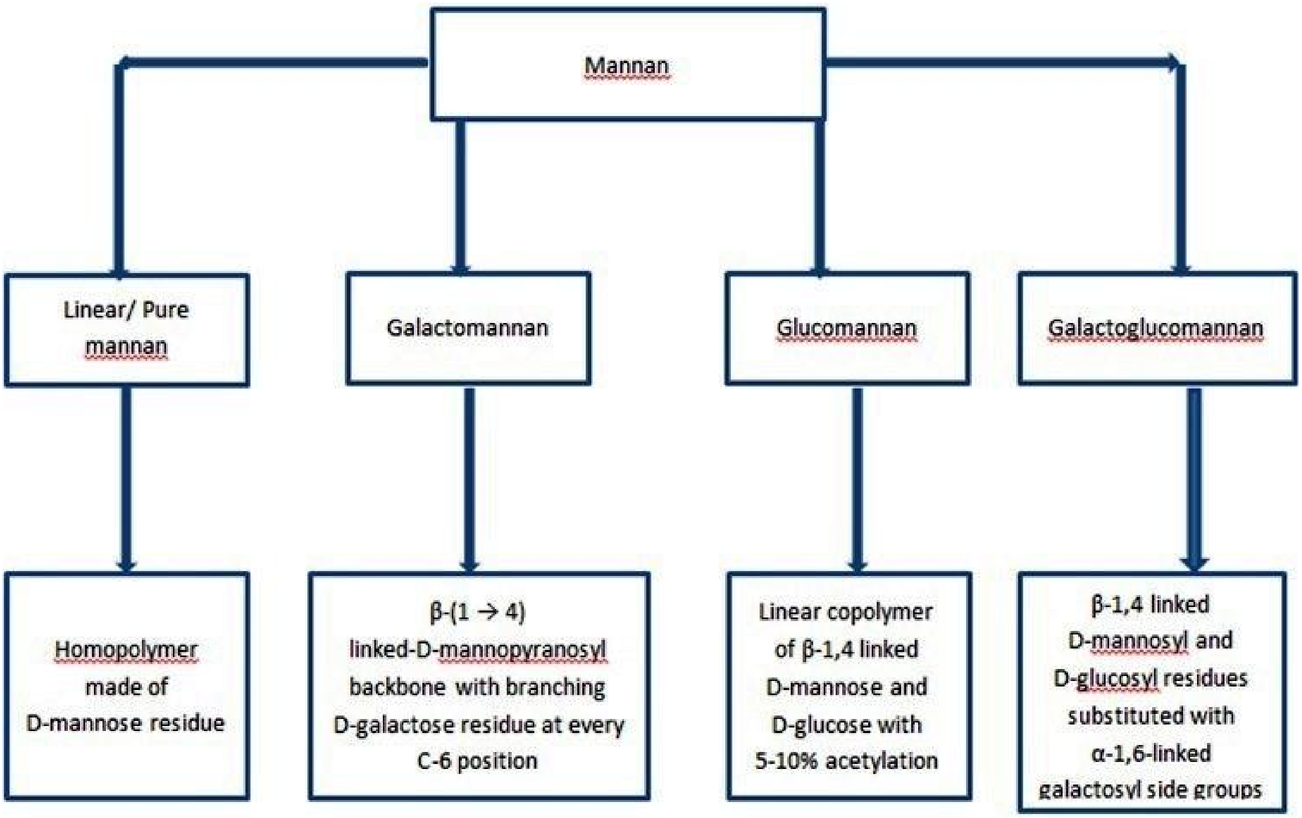

Fig. 2. An illustration of different types of mannan.

Effects of PKC on the intestinal morphology of chicken: There have been various research works conducted with findings stating that supplementation of mannanoligosaccharides (MOS), the main constituent of PKC increased intestinal villi height and crypts depth which has led to better utilization of energy resulting in an improvement in feed conversion ratio (Adeola and Cowieson, 2011). Cheled-Shoval *et al*. (2014) observed that chicks that underwent in-ovo injection of MOS experienced an improvement in the development of their small intestines before and after hatching, had an increase in heights of villi, crypt depth and goblet cells. This increase provided a large surface area for the intestines in its absorption function and decreased the ratio of nutrients absorbed to the number of epithelial cells (vanLeeuwen *et al*., 2004). Carbohydrates and other biomolecules containing carbohydrates can be regarded as true antioxidants which have the ability to look for reactive oxygen species (ROS).

Many works have shown the protective effect of MOS on heat-stressed chickens (Baurhoo *et al.,* 2007; Rehman *et al.,* 2008a,b). When fed to layers, it increased antioxidant activities of egg yolk and liver significantly (Bozkurt, 2012; Rezaei *et al*., 2015). Those feedstuffs that are not easily digested but have the potential of positively affecting the host by way of carefully arousing the growth and/or work of useful bacterial species in the colon are known as prebiotics (Dimitroglou *et al*., 2009; Saad *et al.,* 2013). They are mostly combinations of non-degradable complex saccharides, made up of 3-10monosaccharides that help in promoting the growth, expansion in the number and work of gut microbes. There are various materials that can serve as prebiotics, but dietary fibers such as oligosaccharides form the greatest part (Gourbeyre *et al.,* 2015). Several studies conducted on the function of oligosaccharides have shown that they have the ability of enhancing feed conversion efficiency by way of improving the height of the villi, increasing goblet cells in the jejunum (Choct, 1998; Baurhoo *et al.,* 2007; Mateos *et al*., 2012) and also exert an influence on the immune system of the gut by arousing bacteria metabolism (Gourbeyre *et al.,* 2015; Hosseini *et al*., 2016). The addition of 30% PKC in the feed of laying hens increased the number of beneficial Lactobacilli and suppressed the growth of E. coli in the intestinal tract.

The gastrointestinal tract and microbiota: The gastrointestinal tract (GIT) of animals is an organ system which primarily functions in taking in feed, digesting it, extracting and absorbing nutrients and energy and expelling what is left as feces. The structure of the GIT differs from species to specie each specialized to perform specific function. In poultry, feed eaten moves in a one-way fashion because of the absence of anti-peristaltic movement through the esophagus, crop to the small intestine. Dividing the GIT of monogastric animals into two; upper and lower, the upper digestive tract aside trapping and using energy present in the diet, also delivers materials that have been fermented to the lower digestive tract to nourish the thousands of bacteria residing there (Coles *et al.,* 2010). The gut microbiota plays very important role in animal health and production. The composition and population have a direct correlation and to a large extent exerts positive influence on the immunological and physiological parameters of the host. It also affects the development of the gastro-intestines, biochemistry and nonspecific resistance to infection (Coles *et al.,* 2010).

In the early stage of the growth of chicks, the microbes that inhabit the intestinal tract and their host form a close bond. They play great roles in ensuring energy uptake, utilization and response to anti-nutritional factors (Rinttil¨a, and Apajalahti 2013). There is also a great interaction between the intestinal microbes and their host (Mookiah *et al*., 2014). There are also direct interactions of microorganisms with the lining of the gut which sometimes changes the dynamics of the tract and makes the birds susceptible to infections (Morgan and Bedford, 1995; Sohail *et al*., 2010). This microbiota has one of the highest populations for any ecosystem. There are more than 900 species that play various roles from digestion of food, growth and immunomodulation (Apajalahti *et al.,* 2004). Supplementation of 2.5% PKC reduces the population of Salmonella which is a pathogenic bacteria in the intestine.

The organisms living in the gut mainly degrade complex substrates (NSPs) which requires highly specialized, hydrolytic enzymes (Sergeant *et al.,* 2014, Stanley *et al.,* 2014). One key role of bacteria in the gut is their ability to hydrolyze NSPs which allows other microbes to further ferment them to manufacture short chain fatty acids (SCFA) that can be used by the host for growth and other physiological functions (Tang *et al.,* 2014). The non-digestible components of feedstuffs which cannot be absorbed are oligosaccharides. Because of their indigestible nature, they pass through the gut until they reach the large intestine where they are fermented upon by different microorganisms. These microorganisms like Lactobacillus and Bifidobacterium provide beneficial effect to the animal by producing SCFAs (Bedford and Apajalahti, 2001; Fooks and Gibson, 2001. The acidic environment prevents the survival of gram negative pathogenic bacteria like E. coli, Salmonella and Enterobacter. One of the important roles of colonic microbes is their ability to produce SCFAs. Greater proportion of this products of metabolism are taken from the gut, which makes the host able to derive energy from feed not degraded in the upper part of the digestive tract. SCFA affects transport process and energy transduction in the colon, differentiation (growth and cellular), lipid and carbohydrate metabolism and provision of energy to organs such as kidney, heart, muscle and brain.

The gut microbiota plays important roles in digestion, absorption, and energy metabolism (Rinttil¨a and Apajalahti, 2013). The greatest population of these microbiota are found in the chicken's ceca, which serves as home to numerous microbes involved in preventing colonization, detoxification, recycling, breaking down of indigestible carbohydrates, and absorption of other nutrients. The SCFAs mainly produced by fermentation of microbes, are then absorbed and broken down to produce energy for the host (McWhorter *et al.,* 2009). Conversion of complex carbohydrates to SCFAs in the caecum contributed about 3.5 - 10% of the metabolizable energy (ME) in poultry (J´ozefiak *et al.,* 2004) making it an important source of energy for chickens. At phylum level, Bacteroidetes account for 65% of the total microbiota composition making them the most predominant with the phylum Firmicutes with Proteobacteria making up the remaining 35% (Park *et al.,* 2016; Chen *et al.,* 2018). Shaufi *et al.,* 2015; Sohail *et al*., 2012; Sohail *et al.,* 2015 reported that Firmicutes constitute the highest phylum in the ceca. Several factors such as environmental temperature, broiler chicken used, and feed type can be ascribed the reason for the difference in the population of micoorganisms (Danzeisen *et al.,* 2011; Wei *et al.,* 2013).

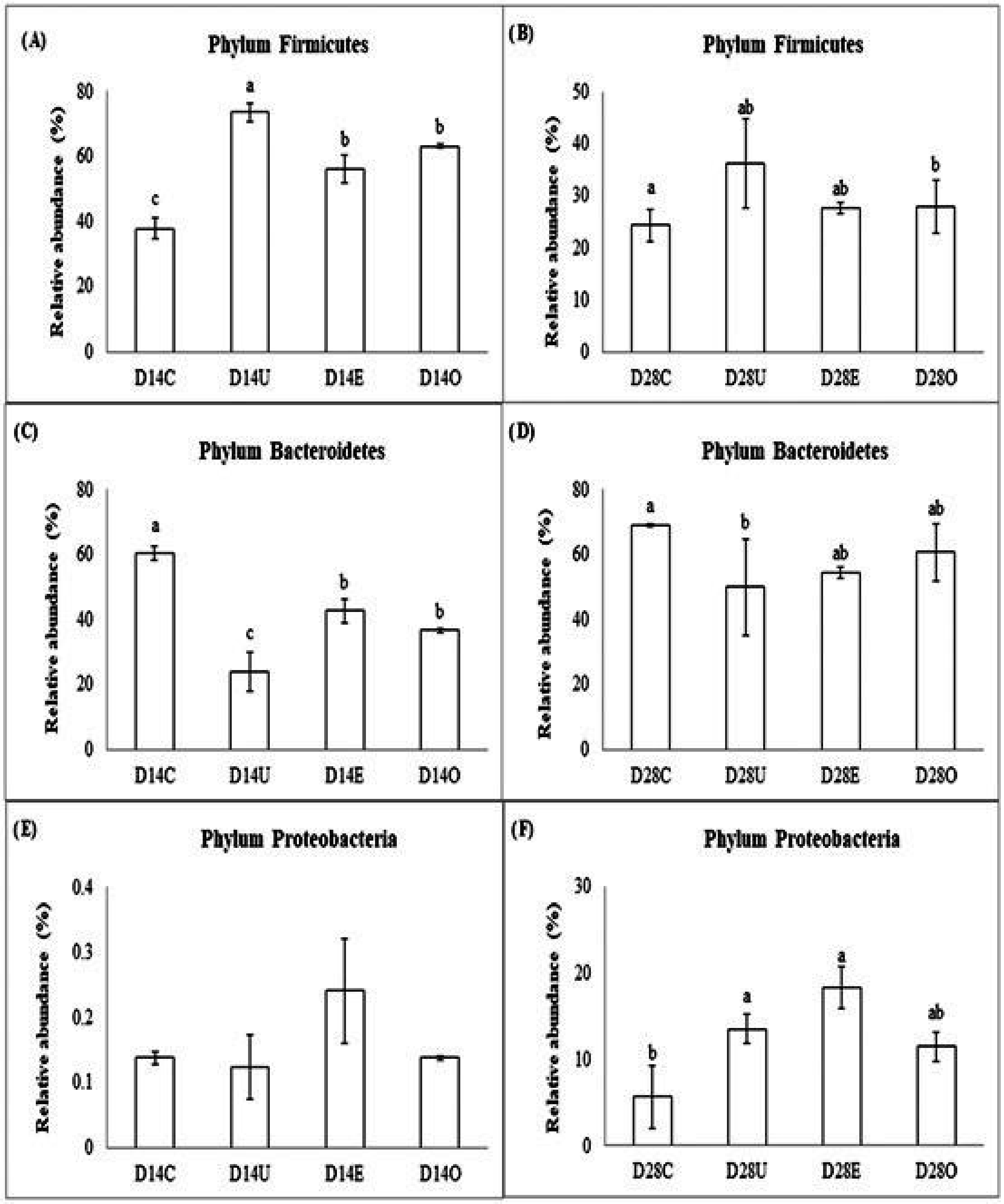

Fig.3. Composition of microbes of broiler fed PKC

Relationship between microbiota composition of the GIT and PKC: The gastro-intestinal tract of chicken can be divided into two; the upper and lower segment. The crop, proventriculus and gizzard make up the upper segment. The main storage organ in the process of digestion in birds is the crop. The crop is an enlarged, muscular pouch close to the gullet. The crop is inhabited by bacteria that help in the fermentation of feed with the Lactobacillus being the dominant (Choct *et al*., 1996). Digestion begins in the true stomach (proventriculus) while the gizzard serves as the grinding mill. The gizzard,has a low pH, ability to ferment ingested feed and serve as a barrier for microbes. The gizzard and crop are predominantly inhabited by Lactobacilli, facultative and microaerophilic bacteria with other species belonging to Clostridiaceae, Enterococcus, Bifidobacterium and Enterobacteriaceae (Rehman *et al*.,2007; Sekelja *et al.,* 2012). The lower segment is made up of the ceca and small intestine which is longer with fixed diameter. It is made up of three segments: duodenum, jejunum and ileum. Starch breakdown and fermentation of lactate is started by several Lactobacillus, Bifidobacterium and

Enterobacteriaceae family that resides in the crop (Amit-Romach *et al.,* 2004). The fermentation of the feed in the crop is anaerobic, makes use of organic molecules to produce lactic acid, ethanol, succinic acid, acetic acid, CO_2, and H_2. Build-up of these end-products helps in the digestion of feed substances that are stored in the crop. The bacteria in the crop help in the fermentation of PKC thereby increasing the nutrient content for the optimal use of the birds. The PKC serves as the substrate for the microorganisms which in turn by their degradation effect help in breaking down the inhibiting factors. Palm kernel cake also helps in increasing the microbial population in the crop and in the maintenance of optimum PH (Amit-Romach *et al*., 2004).

Role of enzymes, nutrient composition of PKC and immune system: There have been growing public concerns about the use of antimicrobial feed additives in animal production, necessitating that better and alternative ways be explored to improve performance in poultry. This has brought about the use of externally supplemented enzymes as a replacement for antibiotics (Morsy *et al.,* 2016, Kholif *et al.,* 2017) to increase the nutrient available to the animals and enhance production. In recent years, addition of exogenous enzymes in the diet of animals has gained ground. This has helped in the breakdown of complex polymeric compounds, leading to improvement in the ability of animals to use the nutrients, better energy conversion rate and reduction in the anti-nutritional factors (Farhangi and Carter, 2007). Several supplemented enzymes like xylanase, protease, β-glucanase, amylase, α-galactosidase, , lipase, phytase and mannanase have been in use in the formulation of diet of chicken for some years now (Bedford and Cowieson, 2012). Fermentation of insoluble fiber in the intestinal regions of animals is more difficult compared to soluble NSPs. This factor has reduced the addition of feedstuffs with low-protein content such as PKC in poultry diet. Adeola and Cowieson, (2011), Bedford and Cowieson, (2012) reported that one good way to increase the nutrient composition of NSPs is by exogenous enzyme supplementation. When PKC undergoes enzymatic treatment, there is a great decrease in the percentage of fiber, increase in the production of soluble sugars and increase in the metabolizable energy (Hanafiah *et al.,* 2017; Chen *et al.,* 2018). This increment surprisingly does not reflect in the growth of broiler chicken (Saenphoom *et al.,* 2013; Navidshad *et al.,* 2016) and was attributed to the fact that the major sugar produced after treatment by enzyme (mannose) is poorly assimilated by broiler chicken (Saenphoom *et al.,* 2013). The poorly assimilated mannose lead to increase in mannose-based compounds in the ceca which eventually lead to alteration in the microbiota composition of the ceca (Chen *et al.,* 2018). The non-digestible food ingredients whose main function is the arousing of growth and/or the work of microbes in the gastro-intestinal tract to promote health of the organism are referred to as prebiotics (Gibson and Roberfroid 1995). They mostly comprise of a wide range of oligosaccharides (OS), with different molecular structures. In the gastro-intestinal tract, they increase the populations of beneficial bacteria like lactic acid bacteria and bifidobacteria (Roberfroid *et al.,* 2010).Diet can improve host resistance to infection by modulating immune function. The role of prebiotics in immunomodulation has been reported by several studies (Collins and Gibson, 1999; Netherwood *et al.,* 1999; Gibson and Fuller, 2000). During fermentation of prebiotics, short chain fatty acids are produced and they function to improve the gut-associated lymphoid tissue and the systemic immune system (Roller *et al.,* 2004; Baurhoo *et al.,* 2007). Gut microbiota also defend against potentially pathogenic competitors and exchange molecular signals between different genera/species and epithelial intestinal cells (Kelly *et al*., 2005; Roberfroid *et al.,* 2010). Mannose, which is a monomer of mannan-oligosaccharides have the ability to inhibit Salmonella infections. Salmonella bounds to mannose through type 1 fimbriae and this reduces pathogen colonization of the gut (Oyofo *et al.,* 1989). Enzymes have the ability to breakdown PKC thereby improving upon the nutrient composition. There is an increase in the population of beneficial microorganisms residing in the gastrointestinal tract of chicken upon supplementation of PKC and this increase in the bacteria population helps in boosting the immune system of chicken.

Conclusion: Palm kernel cake has the ability to improve the development of small intestine of chicks during pre- and post-hatch periods, increasing the villi height, crypt depth and number of goblet cells per villus of poultry but the nutrients are not readily available for use by poultry because of anti-nutritional factors. Further studies should be done on ways to reduce these anti-nutritional factors.

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