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Growth performance and blood serum protein profile of broiler finisher fed with probiotics

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The study which spanned 35 days was carried out to investigate the impact of using probiotics on broiler birds' blood protein profile using the gel protein band development comparison. CRD was used for the experiment. A total of 36 Cobb Vantress birds were raised and fed with probiotics brand (poultry growth enhancer) containing culture solution of *Lactobacillus* spp. i.e milk bacteria, *Bacillus* spp., and *Saccharomyces cerevisiae*. The bird physical parameter such as body weight and feed supplied were weighed and birds were fed with restricted feeding style. The bird fed with probiotics included diet had a significantly higher average daily weight gain than those not fed with probiotics ($p<0.05$) at 40.16 for the treated and 39.09 for the control while the final weight gain on the restricted diet with treatment was not significantly different at 1.13 while those without the treatment was 1.167 and the initial body weight of the control birds was higher than those fed with probiotics in the diet. The electrophoretic protein profile of control and probiotics-fed birds based on a preliminary investigation of 10 randomly selected birds fed with and without probiotics. It was discovered that thicker bands were observed at point for globulins, transferrins, and albumins showing increased level as globulins contain antibiotics for defense, transferrins for oxygen transport, and albumins for greater blood oncotic pressure; this suggests that probiotics had a major impact on the birds' health.

Key words: *growth performance, blood serum, protein profile, broiler finisher, probiotics*

INTRODUCTION

The world's population is expected to surpass 9.7 billion people by the year 2050 (Kuhn *et al.,* 2018), causing food security issues for developing countries in particular. Furthermore, the rising demand for livestock products for animal protein services has increased, making the livestock industry under pressure to produce more often with fewer resources. Regardless of this, the domesticated animals' area is one of the quickest developing farming areas, representing generally 40% of rural agrarian production and giving over 1.3 billion people's means of livelihood and access to food individuals. This development raises worries about the most proficient utilization of assets to create nourishment for people; the impacts of land transformation and escalated use on natural offices and biodiversity preservation; the impacts of ruminant methane creation on environmental change; and the impacts of environmental change-instigated rising temperature on the environment (Popp et al., 2014).

For marginalized and underprivileged individuals in developing countries, livestock is a substantial source of disposable income, and livestock is a vital entrance point into the fight against rural poverty *(Molina-Flores et al., 2020*). Aside from being an excellent source of income and nutrition, in that, it is the best protein source in terms of meat, milk, and other product which helps to build, maintain and replace the tissue in the body i.e muscle, organ, and immune system. Livestock provides manure for use as fuel and fertilizer, as well as draught power. Livestock businesses can also provide inflation-resistant animal assets for insurance and finance (Keeley et al., 2019 and Manyi-Loh, et al., 2018). Intensive production systems are becoming increasingly essential in the cattle industry around the world (Popp *et al.,* 2014). Ruminants can be raised as viable protein sources as they can digest indigestible fibrous feedstuff due to fermentation in the rumen but because of the production of methane which has been associated with global warming they may not be ecologically friendly when raised in a large herd, thereby monogastric such as swine and poultry husbandry is the only viable option for ecologically sustainable animal protein sources and poultry broiler is preferable as they convert feed to animal protein the most with a feed conversion ratio of as less as 1.5 and also within the shortest period possible of 1 month and 15 days i.e 6 weeks which make them a viable option of supply cheapest and fastest animal protein source and the chosen animal for effective production of animal protein to cater for the ever-increasing world population (Moorby & Fraser, 2021).

Despite the numerous advantages of chicken livestock production, it has resulted in two important public health concerns. First, through use of antibiotics as growth promoters in animal nutrition has generated widespread concern, with many countries, including the European Union (EU), forbidding their use due to the danger of building antibiotic resistance in microbes linked to human and animal diseases (Arsène et al., 2021) and Agyare *et al.,* 2018). Furthermore, foodborne zoonotic diseases such as salmonellosis, campylobacteriosis, and pathogenic Escherichia coli infection, to mention just some, are serious public health challenges that can lead to huge economic loss all around the world (Bajagai et al., 2016). Antibiotics have long been used in commercial chicken production as a preventive and growth-promoting chemical. Increased antibiotic use, on the other hand, has resulted in the development of antibiotic-resistant bacteria, production losses, and an increased risk of illness (Van et al., 2020). Furthermore, the widespread use of antibiotics has resulted in an imbalance of gut microbiota, posing health risks as well as antibiotic residues contaminating the environment: Antibiotic growth promoters have been prohibited in several places throughout the world because to these negative consequences (Manyi-Loh et al., 2018). As a result, the entire chicken business has been under pressure to find suitable antibiotic alternatives. In the chicken business, probiotics, prebiotics, and

herbal feed additives, as well as their various combinations, have proven to be viable alternatives to antibiotic growth boosters (Ricke, 2021 & Alagawany *et al.,* 2018). Probiotics are living bacteria that have uni or mixed cultures and have good effects on the host by balancing the indigenous microbial population (Thantsha et al., 2012; Markowiak & Ślizewska, 2017). They improve growth performance (Ezema, 2013), host health, nutrient digestibility, intestinal microflora modulating, and autoimmune immunity development by boosting nutrient digestibility, regulating intestinal microbiota, and enabling innate immunity development (Abd El-Hack et al., 2020); Plaza-Diaz et al., 2019). Bacillus, Lactobacillus, and Saccharomyces are only a few of the microorganisms that are commonly employed in animal and poultry nutrition (Dowarah et al., 2017; Salim et al., 2013).

Probiotics (also known as directly fed microbial) are gaining popularity as an alternative to antibiotic growth promoters (AGP) (Lillehoj et al., 2018). The primary goals of employing probiotics are used in animal feed to improve and sustain an animal's performance (production and growth), as well as to prevent and manage enteric infections. Animal nutritionist uses newly developed probiotic products in response to growing concerns about the subtherapeutic use of AGP in animal feeding and a clearer understanding of the importance of the microbial ecology of the gastrointestinal tract (GIT) identifying animals production (Adhikari et al., 2013). Live microorganisms known as probiotics have been found to boost health. Foods (like yogurt), dietary supplements, and non-consumed cosmetics (like skin creams) are all marketed as probiotics. (Sender et al., 2016). Although bacteria and other microorganisms are commonly thought of as destructive "germs," many microorganisms assist our bodies to function properly. Bacteria found in our intestines, for example, aid in the digestion of food, the destruction of disease-causing germs, and the production of vitamins. Our bodies are home to a large number of microorganisms. Microorganisms outnumber human cells by a factor of ten in the human body. Many of the microorganisms found in probiotic products are similar to or identical to those naturally occurring in our bodies (Wikipedia, 2017).

Probiotics are microorganisms that, when taken, are thought to have health advantages. Ingested bacteria that have been related to human and animal health benefits are now referred to as probiotics. The term gained in popularity after 1980. Élie Metchnikoff, a Nobel winner, popularized the theory by asserting that Bulgarian peasants who consumed yogurt lived longer lives as a result of their way of life. "The intestinal microorganisms' need on food permits us to take efforts to change the flora in our body and replace hazardous microbes with helpful microbes," he argued in 1907. Increased desire for scientific verification of the microbe's alleged benefits has resulted from a huge expansion of the potential market for probiotics (Heak et al., 2018). Although commercial probiotics claim to provide a variety of benefits, including relieving gut pain and boosting the immune system, scientific evidence does not back up these claims.. Yet, according to a systematic review of 320 broiler randomized controlled trials, certain commercially available probiotic bacteria strains from *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*, *Aspergillus oryzae*, *Streptococcus faecium*, and *Torulopsis* spp. when fed in daily doses of 100mg/kg colony forming units (CFU Coliforms and Campylobacter were found to be lower in the probiotic-fed hens than in the control diet-fed chickens (health benefit). Another microbes with useful properties is Yeast (*Saccharomyces cerevisiae*) and its nutritional value with cellular breakdown yielding mannan-oligosaccharides with prebiotic properties. Probiotics are widely considered harmless, but in some situations, they may produce bacteria-host interactions and undesired side effects (Alayande et al., 2020).

MATERIALS AND METHODS

The experiment was performed at the poultry unit, Teaching and Research farm, Obafemi Awolowo University (OAU), Ile-Ife, and the laboratory work was carried out at the Biotechnology laboratory of Animal Sciences, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. It was carried out for 7 weeks where the bird was brooded for 3 weeks and treated on an experimental diet for 4 weeks. Feed was purchased from a commercial feed outlet for selected commercial feed and was divided into two diets for the birds, those with probiotics in their feed at the rate of 1.5ml per kg of commercial feed and animals without probiotics in their feed (Table 1).

Table 1. Proximate composition of experimental diet without probiotics

Items	
Metabolizable Energy(Kcal/kg)	2900
Crude protein (%)	16
Fat/oil(%)	2
Crude fibre(%)	6
Calcium(%)	1
Available Phosphorus(%)	0.40
Lysine(%)	0.88
Methionine(%)	0.35
Salt	0.30

A Completely Randomized Design was used, a total of 36 Cobb Vantress broiler birds were used and randomly allocated to 4 experimental pen units at 9 birds per pen for both treatment groups. Feed and water were supplied ad libitum at 116g per day.

Two weeks before

The environment was cleaned of bushes to prevent vermin invasions. The pens were washed scrubbed and disinfected. The equipment was taken outwash and dried in the sun. Then the pen was fumigated in an air-tight environment with formaldehyde with Potassium to kill germs.

Two days earlier

The equipment was put back inside the pen after it had been opened. To prevent confusion and the entry of predators, the pen's edges that were broken were mended. It is necessary to purchase the feed and water supply.

Before Bird by two hours

To provide heat to the birds outside of the brooding pen before the chicks arrive, an electric lamp and coal pot were added. The hatchery made a point of selecting healthy birds. The Zartech farm provided the bird for purchase. The box was burned as soon as the birds were removed.

Daily routine management

Feed and water were supplied through restricted feeding for the least fat deposition throughout the experiment in the morning. The birds were properly vaccinated as when due vaccinated against New castle and Gomboro (infectious bursar disease) at 7 and 14 days

respectively and 21 and 28th day respectively, appropriate multivitamins (anti-stress) was given to bird during each operation that stresses the bird such as weighing, taking of blood sample and so on. Feeder and drinker were washed each morning before feed and water were supplied to reduce the spread of diseases. The feeder was raised and stones were placed in the drinker to avoid covering themselves with it. Deaths were documented. Litter was replaced every three weeks. Every weekday, the temperature dropped from 37 degrees Celsius to 20 degrees Celsius.

Collection of Data

The collection of data was done at the end of the week, though the first data recorded was weight at the point of stocking of the birds. Individual weights were recorded. Each bird had an average weight of 1.5kg at the end of the experiment. Data was collected on feed intake, body weight, and Mortality. This led to the findings such as the feed intake and feed conversion ratio. Materials used for this laboratory analysis include; Blood of 5,6,7,8 and 10 (two weeks after withdrawal of probiotics) weeks old broiler, Micropipette, test tubes, knife, Pasteur pipette, homogenizer, spatula, test tube rack, water bath, vortex mixer, test tube stopper, petri dish, Bio-Rad Electrophoresis Power Pac Model 200/2.0, weighing scale, Eppendorf tubes, measuring cylinder, centrifuging machine, lightbox, rubber gloves, casting plate, casting stand, tissue paper, measuring syringe(for measuring the recommended level of probiotics) and filter paper, Probiotics (growth performance enhancer: RE3). Preparation of serum: A covered test tube was used to collect whole blood. If the researcher must utilize commercially available tubes, the red-topped tubes should be used. After collecting the complete blood, the blood was left undisturbed at room temperature to clot. It normally takes 15–30 minutes to do this task. In a chilled centrifuge, the clot was extracted by centrifuging at 1,000–2,000 x g for 10 minutes (Ferreira, 1992). After centrifugation, use a Pasteur pipette to quickly transfer the liquid component (serum) into a clean polypropylene tube. During handling, the samples should be kept at 2–8°C. For gel electrophoresis, the centrifuged supernatant containing serum protein was frozen. A minute quantity (10µl) of 1:2 saline diluted serums was even more diluted (1:3) in 40% sucrose solution (to a final1:6). A small drop (50µl) of bromophenol blue an indicator for electrophoretic mobility was added.

Casting gel (10% SDS PAGE) was prepared as follows: Distilled water(2.8ml), 2.5ml of tris. 1.5M HCl, 4.5ml of Acrylamide, 100ml of 10% SDS (Sodium dodecyl sulphate), 50ml of Ammonium Per Sulphate (APS), 10um-Temed (Tetramethylenediamine). After cooling and solidification at room temperature, the water on the top of the gel was drained with a cut filter paper. Stacking gel (4% SDS PAGE) was prepared; Distill water 2.0ml, 0.45ml Acralamide, 0.8ml of 0.5M tris. HCl, 3.5ml of 10% SDS, 30ul of APS, and 10ml of Temed. The stacking gel was added to make up to the brim then it was placed in the refrigerator and the well comb was used to create the well through which the samples were loaded for electrophoresis. A small quantity of 10 microliters (50µg protein) Using a microsyringe, sucrose bromophenol solution was injected into the gel hole. Since this solution is known to denature rapidly within 24 to 28 hours, the process was completed immediately.

Electrophoretic field: The electric voltage was maintained at 180V for the first 30 minutes to allow a direct current of 2-3mA/cm gel, and then the current was lowered to 150V for 45 minutes to enable gel separation (Hammed *et al*., 2011). Staining and destaining of gels. The gel was carefully removed from the equipment following electrophoretic separation and submerged for 18 hours in a staining solution comprised of 40 ml of ethanol, 10 ml of glacial acetic acid, and 0.1 g of newly prepared powdered Coomasie blue diluted in 100 ml of distilled water. When the background was totally clear, the gel was destained in a destaining

solution made up of 60 ml of distilled water, 40 ml of ethanol, and 10 ml of glacial acetic acid (Lan *et al.,* 2004). Protein molecular weight marker. A sample containing several proteins of known molecular sizes is run alongside the test sample in one or more lanes of the gel in order to determine the relative molecular weights (sizes) of the proteins in the gel. Such a set of known molecular weight or protein ladders. The distances each marker protein traveled can be used to create a standard curve. The molecular weights are then extrapolated from the standard based on the distance that the unknown protein traveled. The molecular weights used in kDa are _120= β-galactosidase; ~85= Bovine Serum albumin; ~50= ovalbumin; ~35=carbonic anhydrase; ~25= β-lactoglobulin; ~20= lysozyme.

Protein Profile Analysis: Each gel was scored visually using a lightbox and by looking at its scanned image, which allows us to clearly discern the bands. Where a band is present it is denoted using (1) and the absence of a band is denoted using (0). Also, marker position is of paramount importance to the scoring of bands. The molecular weight position of the bands was compared to establish their molecular weight. Statistical Analysis. As stated, the analysis of variance was used to analyze the data (ANOVA) (Steel and Torrie, 1984). Duncan's multiple range test was used to separate means where substantial differences were found (Duncan, 1955). As a result, a comparison was done between two groups: once-fed probiotics at an inclusion level of 1.5ml to 1kg feed and the control group.

RESULTS AND DISCUSSION

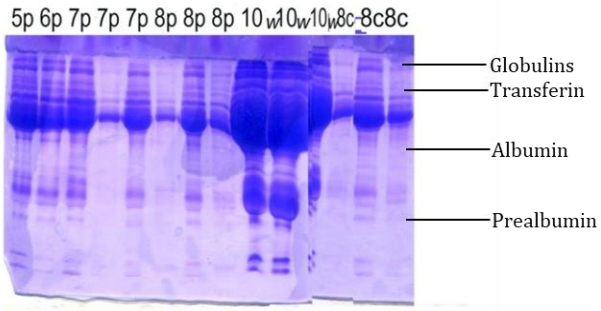
The birds without probiotics in their diet had significantly higher initial body weight which was corrected for by the analysis of covariance for removal of biases on the growth parameter at the end of the experiment. The birds fed with probiotics had significantly higher average daily weight gain, carcass weight, carcass percentage, breast muscle weight, back weight, thigh weight, drumstick weight, head weight and neck weight and they were not significantly different for other parameters and internal weight of organs than those not fed with probiotics $p<0.05$ (Table 2).

Table 2. Growth performance of the probiotics-fed birds

Parameters	Control	Treatment	P(ANOVA)
IBW (g)	472.22[a]	402.78[b]	0.0397
FBW (kg)	1048.12[b]	1182.51[a]	0.3015
MDWG (g)	39.09[b]	42.16[a]	0.0079
Carcass weight	715.47[b]	827.31[a]	0.0005
Carcass Percentage	69.66[a]	69.28[b]	0.0019
Breast Muscle weight	258.68[b]	295.01[a]	0.0005
Back Weight	164.86[b]	185.36[a]	0.0242
Thigh weight	94.0317[b]	112.33[a]	0.0013
Drumstick weight	60.03[b]	73.91[a]	0.0005
Head weight	29.39[b]	31.76[a]	0.0005
Neck weight	45.60b	59.75[a]	0.0198
Wing Weight	92.41	100.68	0.0558
Leg weight	13.32	21.80	0.08225
Fat	1.9374	1.4088	0.6926
Heart	13.6841	11.0000	0.2996
Liver	43.32	38.27	0.6023
Gizzard	54.68	52.88	0.6120

IBW: Initial Body weight, FBW: Final body weight, MDWG: Mean Daily Weight gain

Values within a row with no *common superscripts are significantly different (p<0.05).* All analyses were performed using analysis of covariance. Data were expressed as the mean ± SD. All weight and internal organ are measured in g. As shown in the table above the birds fed with probiotics has significantly different average daily body weight gain when compared to those not fed with probiotics. The carcass percentage was significantly higher for birds without probiotics in diet than those without probiotics in their diet but for the other level of significant different the estimated means for birds with probiotics in their diet was higher than those without probiotics in their diet. The fat content of the meat for both diet was very low but was not significantly different for those fed with probiotics and those without probiotics in their diet showing restricted feeding influenced fat deposition in the birds.



5p: 5th week with probiotics, 6p: 6th week with probiotics, 7p: 7th week with probiotics, 8p: 8th week with probiotics treatment, 10w: 2nd week after probiotics has been removed, 8c: 8th week control

Figure 1. protein profile of the control and probiotics-fed birds

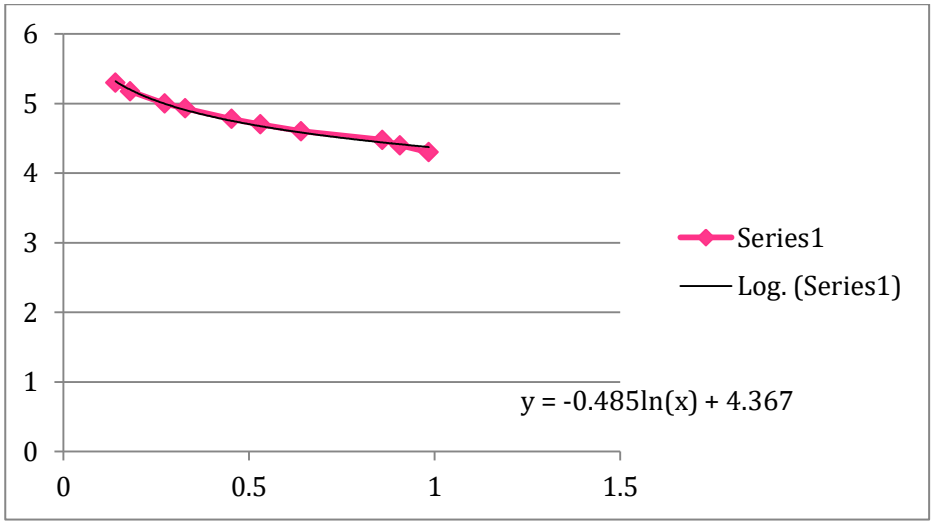


Figure 2. Plot of log of standard of molecular weight to the ratio of distance moved by band

Y = intercept (4.367) ± slope (48.5%) X; where Y is the log of standard of molecular weight and X is the ratio of distance moved by band to gel length.

Plot of log of standard of molecular weight to the ratio of distance moved by band to gel length to generate the relative weight of bands (Figure 2).

From a preliminary analysis of 10 randomly selected birds fed with probiotics and without (Figure 1). It was shown that there was an increase in the number of globulins, transferrins,

and albumins. This indicates that probiotics had a significant effect on the health of the birds since globulins contain antibiotics for defense, transferrins for oxygen transport, and albumins for better oncotic pressure in the blood. The actual contents of each could then be further determined and the increase could be quantified by densitometry and spectrophotometry. As shown in the table above the birds fed with probiotics has significantly different higher average daily body weight gain alongside other carcass characteristics when compared to those not fed with probiotics in agreement with results in agreement with Tang *et al.,* 2017. Also, the internal weights of organs were not significantly different showing it elicited no immune response in fed and non-fed birds with probiotics (Adhikari *et al.,* 2019 & Shabani *et al.,* 2012).

Probiotic-fed birds have a better metabolomic pathway because commensal or symbiotic bacteria compete for food resources, which benefits the host by promoting gut maturation, gut integrity, pathogen antagonisms (competitive exclusion), and immunological regulation. The symbiotic microflora plays a crucial role in gut immunological homeostasis by decreasing inflammation (Shivani *et al.,* 2017). By increasing the number of these bacteria and providing the right substrate for their proliferation and metabolism, the host's nutrient utilization efficiency improves. An increase in the establishment of beneficial gut micro-flora and decrease in pathogenic flora, resulting in increased wastage and also a reduction in gut pH due to the production of organic acids, i.e. volatile fatty acids, resulting in the increased rate of digestion and thus, increased feed and water intake (Abdel-Hafeez *et al.,* 2017). Although previous a few research disagreed with an enhanced growth rate (Xu *et al.,* 2022), some acknowledged that the effect of probiotics is not substantial when combined with a good diet and correct management approach (İncili *et al.,* 2022)) and some postulated increased overall performance and carcass quality (Wang *et al.,* 2020, Ikhimiukor *et al.,* 2022 & Ramos-Vivas *et al.,* 2022).

CONCLUSION

There was increased in carcass characteristics parameter in birds fed with probiotics combination. The broiler chicken fed with probiotics has no quantifiable difference in protein in comparison to those on the control diet. Further studies to quantify the amount of each protein in the sample such as densitometry and spectrophotometry can be done after electrophoresis. Densitometry can be done to quantify the amount of protein of each type in blood and spectrophotometry can be done to determine the total blood protein content. Commercial kits are normally used for human being. Although previous research disagreed with an enhanced growth rate, some acknowledged that the effect of probiotics is not substantial when combined with a good diet and correct management approach.

Abbreviations used

GIT, gastrointestinal tract; FOS, fructooligosaccharides; MOS, mannan oligosaccharides; SPEP, serum protein electrophoresis; HDL, High-density lipoprotein; SDS Sodium dodecyl sulphate; APS, Ammonium Per Sulphate, Temed, Tetramethylenediamine; IBW, Initial Body weight; FWG, Final weight gain; ADWG, Average Daily Weight gain.

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AUTHOR CONTRIBUTIONS

Senior author wrote the Manuscript and performed the experiments; Co-author Prof. O. G. Omitogun gave scientific supervision and edited the Microsoft word document, The Senior Author under the supervision of the coauthor worked on the design of the experiment. Mr Akin gave technical assistance in the laboratory. The work is part of an MSc dissertation submitted as partial requirement for an MSc degree at OAU, Ile-Ife.s

COMPETING INTERESTS

The authors have declared that no conflict of interest exists. The manuscript has not been submitted for publication in other journal.

ETHICS APPROVAL

Not applicable

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