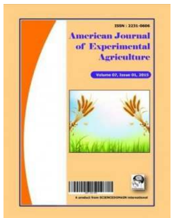

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Influence of Supplemental Levels of Probiotic on Growth Response, Intestinal Microbiota and Carcass Characteristics of Broilers

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of varying levels of commercial probiotic on the performance, microbial status and carcass quality of broiler chickens was investigated in a 42-day experiment between March and April, 2014.

Two hundred (200) one-day-old broiler chickens were randomly allotted to 4 diets with 5 replicates of 10 birds each in a completely randomized design.

Diet 1 was the basal diet with no supplement while diets 2, 3 and 4 were basal diets supplemented with graded levels (200, 400, 600 mg/kg) of probiotic. On day 42, ileal digesta were collected for microbial count and intestinal pH. The weight of carcass primal cuts and visceral organs were also recorded.

There were no remarkable differences observed in the final weight, weight gain and feed conversion ratio of the birds on the experimental diets in both starter and finisher phases. However, significantly higher (P= .05) feed intake (550.00 g/bird) was recorded in birds on diet supplemented with 600 mg/kg probiotics while birds on the basal diet had the least (470.00 g/bird) at day 0-21. Meanwhile between 22-42 days, the highest feed intake (1821.25 g/bird) was recorded for birds on diet supplemented with 400 mg/kg probiotic while the least (1630.00 g/bird) was observed in birds

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fed basal diet. There were no appreciable differences observed in the microbial population in the GIT of birds on the experimental diet except in the gram negative Enterococcus and Lactic Acid Bacteria counts. Birds on 600 mg/kg probiotic dietary supplement had the highest live weight (2142.00 g/bird) which was similar to those on 200 mg/kg and 400 mg/kg probiotics. There were no significant differences recorded for visceral organs and weight of the primal cuts of birds on the experimental diets except for drumstick.

In conclusion, improved growth response and carcass quality were achieved at all the inclusion levels of dietary probiotic without compromising birds' performance.

Keywords: Performance; broilers; carcass quality; microbial population; probiotic.

1. INTRODUCTION

Antibiotic growth promoter in animal production has been practiced for several decades in different parts of the world. Concerns about the potential development of antimicrobial resistance and about transference of antibiotic resistance genes from animal to human microbiota led to the ban of antibiotics as growth promoters in poultry production [1] As a result, it became imperative to look for viable alternatives that could enhance the natural defence mechanisms of animals and reduce the massive use of antibiotics. Such alternative growth promoters include prebiotics, probiotics, enzymes, synbiotics, phytobiotics, plant extracts, acidifiers etc. [2]. They have been found to play an important role in maintaining microbial balance and enhancing gut integrity in poultry.

Probiotics are microbial fed supplements which beneficially affect the animal health by improving its intestinal microbial balance. They are individual microorganisms or groups of microorganisms which have favourable effect on host by improving the characteristics of intestinal micro-flora [3]. Certain species of bacteria, fungi and yeasts belong to group of probiotics. Existing probiotics can be classified into colonizing species (*Lactobacillus sp., Enterococcus sp.* and *Streptococcus sp.)* and free, non-colonizing species (*Bacillus sp.* and *Saccharomyces cerevisiae*) [4]. Their mode of action is based on the inhibition of development of intestinal tract pathogenic bacteria and by decreasing the pH of intestinal digesta, which is a good medium for growth of beneficial microbes. The positive effects of probiotics in animal nutrition have been reported [5,6]. It was therefore the objective of this study to evaluate the effect of graded levels of dietary probiotic on the performance, intestinal microbial load and carcass characteristics of broilers fed corn-soybean diets.

2. MATERIALS AND METHODS

2.1 Experimental Site, Diets and Management of Birds

The experiment was carried out at the Poultry unit of the Teaching and Research Farm University of Ibadan, Nigeria. Two hundred (200) one-day-old unsexed broiler chicks (Abor Acres broiler chickens strain) used for this study were obtained from a reputable commercial hatchery. The birds were reared in a well-ventilated and illuminated poultry house. They were weighed, tagged and randomly allotted to 4 dietary treatments sorted by body weight in a completely randomized design. Each diet had 5 replicates with 10 birds per pen. The experimental diets and fresh water were supplied *ad libitum* for 6 weeks.

Diet 1 was a corn-soybean diet without probiotics, formulated to meet the nutrient requirements [7] for broilers. Diets 2, 3 and 4 were also corn-soybean diet supplemented with 200mg/kg, 400mg/kg and 600mg/kg probiotics (*Lactobacillus sporogenes* + *Saccharomyces cerevisiae)* respectively. The gross compositions of the diets are as shown in Tables 1 and 2.

2.2 Data Collection

2.2.1 Performance indices

Feed intake was calculated as difference between amounts given and left over. The birds were weighed at the end of the starter and finisher phases and values were used to calculate body weight gain and feed conversion ratio.

2.2.2 Digesta collection

On day 42, birds were slaughtered and digesta samples from the terminal ileum (section between Meckel's diverticulum and 2 cm anterior to ileo-caeco-colonic junction) were collected. The contents were flushed with distilled water, pooled according to replicates and frozen for further analysis.

2.2.3 Microbial load and pH of the ileum

Intestinal content from the ileum were separately mixed in a 10 ml pre-reduced salt mediums (Agar) and serially diluted according to the procedure described by [8] to examine the counts of *Escherichia coli* (Rogosa, CM 0627, incubated anerobically 48 hours) *Salmonella spp*, *Enterococcus spp* using MacConkey (MacC) agar, incubated at 39°C for 24 hours. MRS (de Man, Rogosa and Sharp) agar for Lactic acid bacteria incubated at 39℃ for 48 hours and SLA (*Slantez and Bartley*) agar for Enterococci, incubated aerobically for 48 hours at 39°C. The pH of fresh digesta content of the ileum was done by using a Model pH meter (E-HI98128) immediately after collection from the specified sections.

2.2.4 Dissection for carcass traits

At the end of six weeks of feeding trial, two birds per replicate were selected, weighed and slaughtered by cutting the jugular vein. The weights of the primal cuts and visceral organs were taken and recorded. Percentage relative organ weights were calculated as organ weight divided by live weight multiply by 100.

2.3 Proximate Analysis

The proximate composition of each of the diets was determined according to the methods of AOAC [9].

2.4 Statistical Analysis

Data obtained were analyzed using ANOVA of SAS [10] and significant level of P = 0.05 was used. The treatment means were compared using Duncan Multiple Range Test.

3. RESULTS AND DISCUSSION

The results on performance of birds at the starter (d 0-21) and finisher phases (d 22-42) are as shown in Tables 3 and 4. There were no remarkable differences observed in the final weight, weight gain and feed conversion ratio of the birds on the experimental diets in both

Table 1. Gross composition (g/100 gDM) of experimental broiler starter diets (0-21d)

Nutrients	Basal diet	Basal+200 mg/kg probiotic	Basal+400 mg/kg probiotic	Basal+600 mg/kg probiotic
Corn	49.40	49.40	49.40	49.40
Soybean meal	37.00	37.00	37.00	37.00
Wheat	5.00	5.00	5.00	5.00
Soybean oil	4.79	4.79	4.79	4.79
DCP	1.85	1.85	1.85	1.85
Premix	0.16	0.16	0.16	0.16
Limestone	1.35	1.35	1.35	1.35
Methionine	0.10	0.10	0.10	0.10
Lysine	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25
Probiotics	0.00	0.02	0.04	0.06
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
Crude protein	22.85	22.85	22.85	22.85
Energy (kcal/kgME)	3027.36	3027.36	3027.36	3027.36
Ether extract	3.40	3.40	3.40	3.40
Crude fibre	3.83	3.83	3.83	3.83
Calcium	10.26	10.26	10.26	10.26
Total P	7.11	7.11	7.11	7.11
Non-phytate P	4.59	4.59	4.59	4.59
Ca:NPP	2.24	2.24	2.24	2.24

*DCP= Dicalcium phosphate *Composition of premix per kg of diet: vitamin A, 12,500 I.U; vitamin D_3 2,500 I.U; vitamin E, 40 mg; vitamin K_3, 2 mg; vitamin B_1, 3mg; vitamin B_2, 5.5 mg; niacin 55 mg; calcium pantothenate, 11.5 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.025 mg; choline chloride, 500 mg; folic acid, 1mg; biotin, 0.08 mg; manganese, 120 mg; iron, 100 mg; zinc, 80 mg; copper, 8.5 mg; iodine, 1.5 mg; cobalt, 0.3 mg; selenium, 0.12 mg; Anti-oxidant, 120 mg*

Table 2. Gross composition (g/100 gDM) of experimental broiler finisher's diets (22-42d)

Ingredient	Basal diet	Basal+200 mg/kg probiotic	Basal+400 mg/kg probiotic	Basal+600 mg/kg probiotic
Corn	58.23	58.21	58.19	58.17
Soybean meal	30.00	30.00	30.00	30.00
Wheat	5.90	5.90	5.90	5.90
Soybean oil	3.00	3.00	3.00	3.00
DCP	1.20	1.20	1.20	1.20
Premix	0.16	0.16	0.16	0.16
Limestone	1.11	1.11	1.11	1.11
Methionine	0.08	0.08	0.08	0.08
Lysine	0.08	0.08	0.08	0.08
Salt	0.25	0.25	0.25	0.25
Probiotics	0.00	0.02	0.04	0.06
Total	100.00	100.00	100.00	100.00
Calculated analysis (%)				
Crude protein	20.30	20.30	20.30	20.30
Energy (kcal/kgME)	3042.14	3042.14	3042.14	3042.14
Ether extract	3.53	3.53	3.53	3.53
Crude fibre	3.56	3.56	3.56	3.56
Calcium	0.77	0.77	0.77	0.77
Total P	0.57	0.57	0.57	0.57
Non-phytate P	3.28	3.28	3.28	3.28
Ca:NPP	2.36	2.36	2.36	2.36
Ca:P	1.34	1.34	1.34	1.34

*DCP= Dicalcium phosphate *Composition of premix per kg of diet: vitamin A, 12,500 I.U; vitamin D_3 2,500 I.U; vitamin E, 40 mg; vitamin K_3, 2 mg; vitamin B_1, 3 mg; vitamin B_2, 5.5 mg; niacin 55 mg; calcium pantothenate, 11.5 mg; vitamin B_6, 5 mg; vitamin B_{12}, 0.025 mg; choline chloride, 500 mg; folic acid, 1 mg; biotin, 0.08 mg; manganese, 120 mg; iron, 100 mg; zinc, 80 mg; copper, 8.5 mg; iodine, 1.5mg; cobalt, 0.3 mg; selenium, 0.12 mg; Anti-oxidant, 120 mg*

Table 3. Performance indices (g/bird) of broilers fed varying levels of probiotics (d 0-21)

Parameter	Basal diet	Basal+200 mg/kg	Basal+400 mg/kg	Basal+600 mg/kg	SEM
Initial weight	39.12	39.07	39.01	39.10	0.29
Final weight	531.61	538.09	532.42	574.54	75.72
Weight gain	492.49	499.04	493.32	535.46	75.89
Feed intake	470.00^d	535.72^b	480.00^c	550.00^a	0.84
FCR	0.95	1.08	0.97	1.03	1.29

^{abcd}. Mean with different superscript are significantly different (p<0.05), FCR- Feed conversion ratio SEM= Standard. Error of Mean

phases. However, feed intake of birds on dietary supplementation during the starter and the finisher phases was significantly (P<0.05) improved. Birds on diet supplemented with 600 mg/kg probiotic had the highest feed intake (550.00 g/bird) while the least feed intake (470.00 g/bird) was recorded for birds on the basal diet at day 0-21. Meanwhile between 22-42 days, the highest feed intake (1821.25 g/bird) was recorded for birds on diet supplemented with 400 mg/kg probiotic while the least value (1630.00 g/bird) was observed in birds on the basal diet.

These results corroborate the findings of [11,12] who reported no remarkable effect of probiotic supplementation on performance of broilers. This was supported by [13] who observed that the inclusion of probiotic and symbiotic in a corn-soyabean meal-based diet did not improve feed intake, feed conversion ratio and protein intake of turkey poults at the grower phase. In contrast, [14,15] asserted increased weight gain in birds upon feeding probiotic supplemented diet. According to [16,4], it was postulated that different results accrued in probiotic application to poultry diets probably depend on many factors, among which are species composition of probiotic, administration levels, application methods, overall diet composition, bird age and environmental factors.

Results on microbial load and intestinal pH of birds on graded levels of dietary probiotic are shown on Table 5. There were no considerable differences observed in the population of *Escherichia coli,* Salmonella and gram positive Enterococcus and Streptococcus in the GIT of birds on the experimental diet. Similarly, the experimental diet had no appreciable effect on the digesta pH of birds. However, gram negative Enterococcus and Lactic Acid Bacteria (LAB) counts in birds were influenced by the dietary supplementation. Highest population of gram negative Enterococcus was observed in birds on the basal diet and those on 200 mg/kg and 400 mg/kg probiotic while lowered Enterococcus count (5.18×10^4 cfu/ml) was recorded in birds on 600 mg/kg probiotic supplemented diet.

Similarly, LAB population in birds on the basal diet, 200 mg/kg and 600 mg/kg probiotic were identical when compared to those on 400 mg/kg probiotic supplementation which were not significantly different from birds on 600 mg/kg probiotic supplemented diet.

The present results on microbiota showed an effective modulation of intestinal microflora and significant inhibition of the pathogenic microbes as a result of dietary probiotic supplementation. Most of the pathogens grow in a pH close to 7 or slightly higher. In contrast, beneficial microorganisms live in an acidic pH (5.8-6.2) and compete with pathogens. According to [17], there was reduction in intestinal pH in the GIT of broilers fed probiotic supplemented diet. Meanwhile, the intestinal pH (5.18-6.12) recorded in birds fed with dietary supplements was similar to the recommended acidic medium for the growth and balance of beneficial microbes and for maintenance of gut health. The lower pH recorded in probiotic group could probably be attributed to the fermentative activity of lactic acid bacteria on the carbohydrate producing more lactic acid.

Carcass characteristics of birds fed supplemental levels of probiotic are presented in Table 6. Birds on 600 mg/kg probiotic dietary supplement had the highest live weight (2142.00 g/bird) which was similar to those on 200 mg/kg and 400 mg/kg probiotics. There were no significant differences recorded for visceral organs and relative weight of primal cuts of birds on the experimental diets except for drumstick. It was observed by [18] that supplemental probiotic had no effect on weight of internal organs. This was similar to the report of [13] who averred that supplementation of probiotic and symbiotic to turkey diets had no noticeable effect on the relative weight of bursa of fabricius, liver, heart, spleen, pancreas and gizzard.

Table 4. Performance indices (g/bird) of broilers fed varying levels of probiotics (d 22-42)

Parameter	Basal diet	Basal+200 mg/kg	Basal+400 mg/kg	Basal+600 mg/kg	SEM
Initial weight	531.61	538.09	532.42	574.54	75.72
Final weight	1444.76	1432.53	1537.75	1539.26	198.24
Weight gain	913.15	894.44	1005.33	964.75	88.21
Feed intake	1630.00^c	1740.00^b	1821.25^a	1740.00^b	7.72
FCR	1.79	1.95	1.81	1.80	1.32

Error of Mean

Table 5. Microbial load ($cfu\times10^4$/ml) and intestinal pH of broilers fed graded levels of probiotics

Parameter	Basal diet	Basal+200 mg/kg	Basal+400 mg/kg	Basal+600 mg/kg	SEM
E-coli	8.24	7.63	6.42	5.74	0.71
Salmonella	4.88	6.87	4.03	4.92	1.13
Enterococcus^0	6.82^a	6.59^a	6.81^a	5.18^b	0.40
Enterococcus^1	6.50	6.94	6.50	5.35	0.66
LAB	7.08^a	7.05^a	5.85^b	6.65^{ab}	0.34
Streptococcus	6.36	6.14	6.98	7.40	1.23
pH	5.90	5.18	6.12	5.40	0.29

Enterococcus ^{0}; gram negative enterococcus isolated by MacConkey agar, Enterococcus^1; gram positive enterococcus isolated by SLA agar, LAB; Lactic acid bacteria

Table 6. Carcass characteristics (g/b) and relative organ weights (%) of broilers fed graded levels of probiotics

Parameter	Basal diet	Basal+200 mg/kg	Basal diet+400 mg/kg	Basal+600 mg/kg	SEM
Live weight	1883.00^{b}	1981.80^{ab}	1986.00^{ab}	2142.00^{a}	91.10
Dressed weight	1425	1535	1565	1605	95.00
Head	2.45	2.47	2.61	2.48	0.10
Neck	4.58	4.21	4.22	4.13	0.42
Breast	17.42	17.67	17.31	18.3	0.67
Back	13.92	14.15	13.27	13.72	0.66
Wings	7.18	7.60	7.27	7.29	0.27
Drumstick	9.23^{a}	8.01^{b}	8.16^{ab}	8.89^{ab}	0.50
Thigh	8.52	9.31	8.88	8.99	0.45
Shank	3.78	3.90	3.84	3.95	0.18
Gizzard full	3.31	3.25	3.22	3.11	0.20
Gizzard empty	2.17	2.20	2.04	2.14	0.20
Lung	0.49	0.47	0.43	0.46	0.04
Liver	2.27	2.13	2.03	2.05	0.13
Spleen	0.11	0.09	0.10	0.10	0.02
Heart	0.49	0.50	0.49	0.51	0.04
BOF	0.17	0.21	0.17	0.20	0.03

**Means on same row with different superscripts are significantly (P<0.05) different; LW= live weight; DW= dressed weight; BOF= bursa of fabricius*

4. CONCLUSION

The result of the study shows that **i**mproved growth response and carcass quality was achieved at all the inclusion levels of dietary probiotic without compromising birds' performance. Probiotic used in this study conferred health benefits on the birds by improving their intestinal microbial balance.

DECLARATION

The manuscript was previously presented in the following conference.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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