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Effect of Dietary Combination of Probiotics and Prebiotic on Performance Indices and Haematological Parameters in Pearl Guinea Fowls (*Numida meleagris*)

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ABSTRACT

The study aims to evaluate the effects of a commercial probiotics and prebiotic combination on performance indices and haematological parameters of guinea fowls. A total of forty (n=40) day-old pearl guinea fowls raised to 12-week-old were used for the study. The fowls were wing-banded, weighed individually and randomly allocated into two groups of 20 fowls each, with each having two replicates. Experimental fowls were fed grower diet supplemented with 0.5 g of probiotics and prebiotic combination per kg feed for 8 weeks, while the control fowls were fed a grower diet that was not supplemented. Feed consumption and water intake were recorded daily, chest circumference, body weight, percentage weigh gain and feed conversion ratio were measured weekly, while blood samples were collected in the last week of the experiment to evaluate the haematological parameters. All birds were kept under similar environmental, managerial and hygienic conditions. The results revealed that experimental fowls had significantly ($P < 0.05$) higher feed consumption, water intake, total protein, packed cell volume (PCV), red blood cell (RBC) count and haemoglobin (Hb), but lower total leucocyte count (TLC) compared with control. No significant difference was observed in other haematological parameters and performance indices between the two groups. In conclusion, 0.5 g/kg diet of prebiotics and probiotic combination improved erythropoiesis and nutritional status of guinea fowls; however, the results of performance indices suggest that this dose may not be optimum, thus necessitating the need for more research using other dosages.

Keywords: Feed intake, water intake, chest circumference, feed conversion ratio, total protein

Guinea fowl is the common name of the seven species of gallinaceous birds of the family *Numididae*, which are indigenous to Africa, but have been domesticated in other countries of the world (Moreki 2006). In Nigeria, the two main species of guinea fowl that have been described include; the crested guinea fowl (*Gutteraedopuardi*) which is found in the rain forest zone and the pearl or helmeted guinea fowl (*Numidameleagris*) found in the savannah zone, with the latter being the most predominant (Ikani and Dafwang 2004). Guinea fowl farming is popular amongst smallholder farmers in Africa (Nwagu and Alawa 1995). Over the years, however, guinea fowl production is gaining importance in the Nigerian poultry industry, and a shift from the extensive management system, commonly practiced by some Fulani livestock farmers to semi-intensive and intensive rearing system has been observed. This necessitates the need for extensive and intensive studies into every aspect of poultry production that may contribute to the future improvement of this species (Onyeanusi 2007). The interest in guinea fowl production is probably due to the attractive plumage, game-type flavour of its meat, the high meat to bone ratio of its carcass (Embury 2001) and high patronage of its eggs. Also, the ability of guinea fowls to protect themselves against predators and their greater resistance to common poultry parasites and diseases makes production relatively less challenging (Mafuvadze *et al.* 2008). Young guinea fowls of 0 – 12-week-old are called guinea keets. When the guinea keets are 12-week-old, they are either managed to become layers, through feed restriction or exposed to continuous feeding pattern to be finished as table birds (Moreki 2006; Ikani and Dafwang 2004).

The term probiotics is a preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects to the host (Schrezenmeir and de Vrese, 2001; Aluwong *et al.* 2013). Whereas, prebiotics is defined as a non-digestible feed ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon (Schrezenmeir and de Vrese, 2001). Probiotics and prebiotics have been used to improve animal productivity, enhance the immune status, control enteric diseases, reduce contamination of animal products and upgrade the health and nutritional statuses of animals (Meimandipour *et al*, 2010; Aluwong *et al.* 2013; Park and Kim, 2014).Combination of probiotic and vitamin C has been reported to improve weight gain and reduce cost of production in rabbits (Smitha*et al.* 2014). However, decrease in body weight and abdominal fat was observed in Pekin ducks supplemented with Kefir (a natural probiotic; Kandir and Yardimci, 2015). Previous research has shown that haematology is important in evaluating the health and nutritional statuses of an animal (Nalubamba *et al.* 2014; Habibu *et al.* 2014) and administration of both probiotics and prebiotics may influence the haematological parameters of chickens (Al-Saad *et al.* 2014; Sahir *et al.* 2014). Also, the use of heterophil-lymphocyte (H:L) ratio as a sensitive haematological biomarker relevant to immune function has been well established with probiotics markedly decreasing the values of H:L in chickens (Rajalekshmi *et al.* 2014; Sahir *et al.* 2014).

Table 1: Nutrient composition of the grower diet fed to guinea fowls (Used) and composition of diet recommended for guinea fowls (Recommended)

Nutrients	Composition	
	Used	Recommended
Crud protein (%)	15.00	15.00
Fat (%)	3.6.00	-
Crude Fibre (%)	8.60	-
Calcium (%)	1.10	1.00
Available Phosphorus (%)	0.40	0.40
Methionine (%)	0.37	0.34
Lysine (%)	0.70	0.82
Metabolizable Energy (Kcal/Kg)	2500.00	1130.00

Sources: Used (Hybrid Feed®, Kaduna, Nigeria) and Recommended (Moreki, 2006)

Despite the importance of guinea fowl in poultry farming, research is generally focused on the chickens and findings extrapolated to guinea fowls. In most of the previous research on probiotics and prebiotics, focus had been on chickens leading to a dearth of information on the influence of probiotics and prebiotics in guinea fowl production. This study was aimed at evaluating the effects of a commercial probiotics and prebiotic combination on performance indices and haematological parameters of pearl guinea fowls.

Table 2: Chest circumference (CC) and body weight (BW) in guinea fowls supplemented with probiotic and prebiotics combination (Experimental) and control

	Initial	Final
CC (cm)		
Control	27.45 ± 0.53	34.54 ± 0.31
Experimental	28.20 ± 0.63	34.54 ± 0.55
BW (kg)		
Control	0.90± 0.04	1.36 ± 0.03
Experimental	0.89± 0.06	1.39 ± 0.04

There was no significant difference (P>0.05)

MATERIALS AND METHODS

Guinea fowl management and experimental design

The study was carried out in the Livestock Unit of the Samaru College of Agriculture, Division of Agricultural Colleges, Ahmadu Bello University, Zaria. A total of forty (n=40) unsexed day-old pearl guinea fowls raised to 12-week-old were used for the study. The fowls were wing-banded, weighed individually and randomly allocated into two groups (experimental and control) of 20 fowls each with each group having two replicates. The birds were housed in deep litre pen and fed Hybrid® grower feed which have nutritional composition similar to that recommended for growing guinea fowls (Table 1; Moreki, 2006). The experimental and control fowls were kept in separate pens measuring 4 m long and 3 m wide each. The probiotics (*Lactobacillus*, *Streptococcus*, *Bifidobacter*, and *Candida albicans*) and prebiotic (*Mannanoligosaccharide*) combination commercially identified as T.G.I.® (Polchem Hygiene Laboratories PVT,

India) was used as feed supplement in the current study. During the experiment that lasted for 8 weeks, control fowls were fed grower diet that was not supplemented with probiotic and prebiotic, while the experimental fowls were fed grower diet supplemented with 0.5g of probiotic and prebiotic combination per kg feed according to the manufacturer's recommendation in chickens. Feed and water were provided *ad libitum* to both groups of fowl. Both groups of birds were kept under the same environmental, managerial and hygienic conditions.

Table 3: Mean (±SEM) values of haematological parameters in guinea fowls supplemented with probiotic and prebiotics combination (Experimental) and control

Parameters	Control	Experimental
Total protein (g/dL)	5.56 ± 0.11	6.10 0.41*
PCV (%)	31.18 ± 0.42	36.23± 1.80*
RBC(10^6/µL)	2.31 ± 0.02	2.75 ± 0.14*
Haemoglobin (g/dL)	17.33 ± 4.13	19.9 ± 19.05*
MCV (fL)	135.5 ± 0.81	137.5 ± 1.62
MCH (pg)	75.15 ± 1.65	76.82 ± 1.20
MCHC (g/dL)	55.52 ± 1.02	55.98 ± 1.02
TLC(10^6/µL)	4.87 ± 0.34	6.12 ±0.40*
Lymphocytes (%)	83.89 ± 1.26	84.09 ± 2.26
Heterophils (%)	15.89 ± 1.33	14.36 ± 1.92
Monocytes (%)	1.20 ± 0.11	1.67 ± 0.32
H:L	0.18 ± 0.03	0.18 ± 0.03

Values with asterisk (*) indicate significant difference (P<0.05) compared to control

Measurement of performance indices and haematologic parameters

Performance indices such as daily feed consumption, daily water intake, chest circumference, body weight, percentage weight gain and feed conversion ratio were measured. The weekly body weight and chest circumference were measured every other week. Body weight was obtained by weighing the fowls individually using Metlar MT-5000D Electronic Balance. Percentage weight gain was then calculated from the initial and final live weights. The amount of feed and water given to each group of birds was determined daily and the amount left was also recorded the following day to obtain the daily feed consumption and water intake, respectively. The feed conversion ratio was then calculated using the expression: feed consumed/ weight gain.

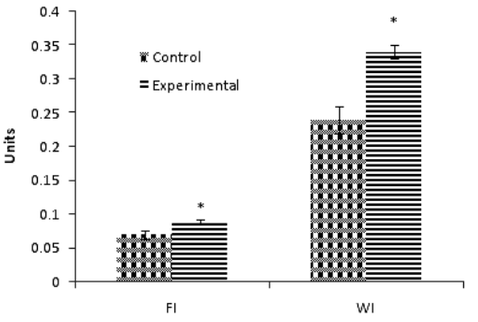

Fig. 1: Mean values of weekly feed intake (FI; kg/bird) and water intake (WI; L/bird) in guinea fowls supplemented with probiotic and prebiotics combination (Experimental) and control. Values with asterisk (*) indicate significant difference (P<0.05) compared to control

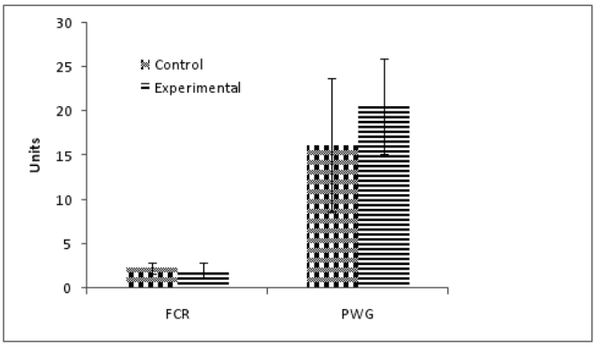

Fig. 2: Mean values of weekly feed conversion ratio (FCR) and percentage weight gain (PWG; %) in guinea fowls supplemented with probiotic and prebiotics combination (Experimental) and control. There was no significant difference (P>0.05)

Blood samples were collected in the last week (week-8) of the experiment and haematological parameters which include: blood total protein, erythrocyte parameters, erythrocytic indices, leucocyte parameters and heterophil-lymphocyte ratio (H:L) were determined. Blood samples (5 mL) were obtained between 08:00 a.m. – 10:00 a.m. through the wing vein into vacutainer tubes containing potassium ethylenediamine-tetraacetic acid (K_3EDTA). The blood total protein (TP) was determined using a hand-held clinical refractometer (Atago®, Master Refractometer, Japan). Packed cell volume (PCV) was measured using the microhematocrit method as described by Jain (1986).

Counting of erythrocytes (RBC) and leukocytes (TLC) was done manually using a haemocytometer as described by Dacie and Lewis (1995). Haemoglobin concentration (Hb) was determined using a haemoglobin meter (XF-1C Hemoglobin meter, China) and the erythrocytic indices were then calculated.

Data analysis

The data obtained were expressed as mean (± SEM) and subjected to student's *t-test* for comparison between groups. The statistical package used was GraphPad Prism version 5.01 for windows (2007) from GraphPad software, San Diego California, USA (www. graphpad.com). Values of $P < 0.05$ were considered significant.

RESULT

Table 2 and Figure 1 and 2 show the mean values of performance indices in probiotic and prebiotic treated and control guinea fowls. The experimental fowls consumed significantly ($P<0.05$) more feed and water than the control fowls (Figure 1). There was no significant difference ($P > 0.05$) in chest circumference and body weight between experimental and control fowls throughout the study period (Table 2). Although not statistically significant ($P>0.05$), experimental fowls had lower feed conversion ratio, but higher percentage weight gain compared with control fowls (Figure 2).

Table 2 shows the mean value of haematological parameters in experimental and control fowls. The TP, PCV, RBC and Hb were significantly higher ($P<0.05$) in experimental fowls compared with control. Other erythrocyte parameters did not differ significantly. The TLC was significantly lower in experimental fowls compared with control. Values of the differential leucocyte counts which include percentage lymphocyte, heterophil, monocytes counts and H:L did not change significantly.

DISCUSSION

Values of body weight obtained in the present study were within the range reported by Adjetey *et al*. (2014) in adult Ghana indigenous guinea fowls. A progressive increase in performance parameters was observed from week 1 – 8 of the experiment. Despite the significantly high feed intake in the treated fowls, there was only a slight increase in chest circumference, percentage weight gain and an insignificant decrease in feed conversion ratio when compared to control. According to the study of Oso *et al*. (2014), manan oligosaccharide (MOS) supplementation did not affect feed intake, weight gain and feed conversion ratio in young guinea fowls. In older fowls, however, dietary supplementation with MOS increased weight gain in fowls fed ground millet and reduced feed intake in fowls fed whole millets with no effect on feed conversion ratio. Generally, in chickens, both probiotics and prebiotics have been demonstrated to either increase weight gain and reduce feed conversion ratio (Aluwong *et al.* 2013; Lan *et al.* 2003; Shahir *et al.* 2014) or have no effect on this performance indices (Kumprechtova *et al.* 2000; Calik and Ergun, 2015; Boguslawska-Tryk *et al.* 2015). Supplementation of probiotic and prebiotic to two separate groups of chicken showed that the prebiotic treated chickens had higher feed intake and weight gain, but lower feed conversion ratio, while the probiotic treated chickens had higher feed intake with no effect on weight gain and feed conversion (Shahir *et al.* 2014). The slightly higherchest circumference and percentage weight gain, but slightly lower feed conversion ratioin treated group suggests that probiotics and prebiotics combination may have the potential of increasing the performance of guinea fowls. The high water intake in the probiotic and prebiotic treated fowls is probably due to increased metabolic demand for water resulting from high feed intake. The differences in results between the present and previous study may be due to the combined effect of probiotics and prebiotic, species difference, duration and age the birds when the agent was administered.

Blood total protein has been shown to be high in birds fed diet with improved plane of nutrition, while a reduction in blood total protein has been associated with low dietary protein utilization (Schalm *et al.* 1975; Church *et al.* 1984; Liu *et al.* 2015). The present study recorded higher values of blood total protein in treated fowl compared with control. Thus, suggesting an improvement in the nutritional status of the treated birds. This is in agreement with the finding of Oso *et al*. (2014) who reported a higher blood total protein in guinea fowls supplemented with MOS irrespective of age. On the contrary, supplementation of probiotic and prebiotic to two separate groups of chickens had no effect on blood total protein (Shahir *et al.* 2014). This suggests species difference in the response of bird to treatment with prebiotics.

Values of blood cellular components recorded in the present study are within the normal range reported by Adedibu *et al*. (2014). In the present study, dietary supplementation with a combination of probiotic and prebiotic markedly increased the PCV, RBC and Hb of guinea fowls. In turkey, probiotic supplementation resulted in an increase in PCV, RBC and Hbwhile, supplementation with prebiotic (MOS) caused no change in this erythrocyte parameters (Cetin *et al.* 2005). In chickens, however, probiotic supplementation resulted in an increase in PCV, with no effect on RBC and Hb, while prebiotic supplementation had no effect on this erythrocyte parameters (Al-Saad *et al.* 2014). On the other hand, some studies have also shown that probiotic supplementation has no effect on haematological parameters of chickens (Alkhalf *et al.* 2010). The high value of serum total protein, PCV, Hb and RBC may suggest an improved nutritional status in the fowls supplemented with a combination of probiotics and prebiotic. This may be due to enhanced digestion and absorption of nutrient in the treated fowls. Probiotics are known to stimulate rapid colonization of the gastrointestinal tract (GIT) by beneficial bacteria through competitive exclusion. This consequently results in improvement in the absorption of nutrients in the intestine of the birds (Alkhalf *et al.* 2010; Park and Kim, 2014). Prebiotics on the other hand, enhance the production of short chain fatty acids which prevent the growth of pathogenic microbes (*E. coli*, *Salmonella spp.*, *Clostridium spp*. and *Campylobacterspp*.; Boguslawska *et al.* 2015; Meimandipour *et al*, 2010; Arsi *et al.* 2015) by lowering the pH to create an unsuitable environment around the pathogens (Savage, 1991). This favours the selective colonization of the gastrointestinal tract (GIT) by beneficial microbes (lactobacillus spp.; Zhu *et al.* 2015; Biggs *et al.* 2007).

Unlike prebiotics supplementation in chickens (Shahir*et al.* 2014), supplementation of probiotics have been shown to improve immune status (increased antibody titre) against Newcastle disease vaccine and also increase the count of monocytes with no significant effect on TLC, lymphocyte and heterophil counts (Zulkifli *et al.* 2000; Al-Saad *et al.* 2014; Shahir *et al.* 2014; Park and Kim, 2014).In the present study, however, TLC was lower in treated fowls than control. The low TLC in treated fowls is probably due to reduction in the challenge posed to the immune system by pathogenic microbes in the GIT, since probiotic and prebiotic suppress the growth of pathogenic microbes in the GIT. Moreover, the values of H:L, lymphocyte, heterophil and monocyte counts were not affected by supplementation with probiotics and prebiotic combination. Supplementation of probiotic and prebiotic to two separate groups of chickens had no effect on TLC, lymphocyte, heterophil and monocyte counts (Al-Saad *et al.* 2014; Shahir *et al.* 2014), in addition, however, the chickens treated with probiotic had lower H:L (Shahir*et al.* 2014) compared with control. In the study of Capcarova *et al*. (2008), a non-significant decrease in TLC was observed in the two groups of turkey supplemented with graded doses of probiotic. Species difference and the combined effects of probiotics and prebiotic may account for this different result observed in TLC between the present study and previous study in chickens.

CONCLUSION

Dietary incorporation of commercial prebiotics and probiotic combination at 0.5 g/kg diet improved feed and water intake, erythrocyte parameters and TP, but has little effect on weight gain in 12-weeek-old guinea fowls. The results of performance indices and the decrease in TLC suggest that this dosage (0.5 g/kg diet) may not be optimum, thus necessitating the need for more research using other dosages or younger fowls.

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