Evaluation of the probiotic potential of the supernatant of fermented maize (*Omidun*) on growth performance, survivability, and haematology in broiler chickens

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Abstract

This study aimed to assess the use of supernatant of fermented maize (SFM) (Omidun) as a potential source of probiotic on growth, survival rate, and haematology in broiler chickens. After being acclimatized for 7 days, 150 eight-day-old Cobb 500 broiler chicks were randomly divided into five treatment groups of 30 birds, with each group having three replicates. All the birds were fed a commercial diet, group A served as the negative control and group B was given a commercially prepared probiotics (Beta probiotics) containing Lactobacillus spp., (2.7 $\times 10^6$ CFU/mL), Bacillus cereus (6.5 $\times 10^6$ CFU/mL), Bacillus subtilis (7.8 $\times 10^6$ CFU/mL), protease, (250,000 IU), lipase (70,000 IU) and amylase (500,000 IU) via the drinking water, while groups C, D and E were given SFM at 10, 5 and 2.5 mL/L drinking water respectively. The experiment lasted for six weeks. Data were collected weekly, analyzed using one-way repeated measure ANOVA and presented in tables. The treatment revealed that no significant difference (p>0.05) was observed in the growth and haematological parameters of studied birds. Although, the birds on the varying concentrations of SFM did not significantly differ from the control groups, there was no negative effect on the growth performance, livability and haematology of broiler chickens.

Keywords: Broiler chicken, Fermented maize supernatant, Growth, Haematology, Probiotics
Running title: Broiler chickens' productivity with *Omidun* supplementation

Évaluation du potentiel probiotique du surnageant de maïs fermenté (Omidun) sur la performance de croissance, la survie et l'hématologie chez les poulets à griller

Résumé

Cette étude visait à évaluer l'utilisation du surnageant de maïs fermenté (SMF) (Omidun) comme source potentielle de probiotique sur la croissance, le taux de survie et l'hématologie chez les poulets de chair. Après une acclimatation de 7 jours, 150 poussins de Cobb500 âgés de huit jours ont été répartis aléatoirement en cinq groupes de traitement de 30 oiseaux, chaque groupe ayant trois répliques. Tous les oiseaux ont reçu un régime commercial, le groupe A servant de contrôle négatif et le groupe B recevant des probiotiques préparés commercialement (Beta probiotics) contenant Lactobacillus spp. (2,7 × 10^6 CFU/mL), Bacillus cereus (6,5 × 10^6 CFU/mL), Bacillus subtilis (7,8 × 10^6 CFU/mL), protéase (250 000 IU), lipase (70 000 IU) et amylase (500 000 IU) par l'eau de boisson, tandis que les groupes C, D et E recevaient respectivement du SFM à 10, 5 et 2,5 mL/L d'eau de boisson. L'expérience a duré six semaines. Les données ont été collectées chaque semaine, analysées à l'aide d'une ANOVA à mesures répétées à un facteur et présentées dans des tableaux. Le traitement a révélé qu'aucune différence significative (p > 0,05) n'a été observée dans les paramètres de croissance et hématologiques des oiseaux étudiés. Bien que les oiseaux ayant reçu les différentes concentrations de SMF ne différaient pas significativement des groupes *de contrôle, il n'y avait aucun effet négatif sur la performance de croissance, la viabilité et l'hématologie des poulets à griller.*

Mots-clés : poulets à griller, Surnageant de maïs fermenté, Croissance, Hématologie, Probiotiques

Introduction

The upsurge in the consumption of chicken meat has been attributed to the belief that it is a healthy source of animal protein, containing over 20% protein with more than 40% of the total amino acids being essential ones (Haščík *et al.*, 2020). Chicken meat is lower in saturated fatty acids than other meat and high in unsaturated fatty acids (Kim *et al.*, 2015); it is cheap and easily available; it can be easily processed into other products, and its consumption has been recorded as having virtually no religious or cultural restrictions (Dalle Zotte *et al.*, 2020). To keep up with this increasing demand for poultry meat, there is a need to scale up its production, which has led to the use of various forms of antibiotic growth promoters (Verstegen and Schaafsma, 1999; Cummings and Macfarlane, 2002). In poultry production, antibiotics are usually supplemented in the diets at sub-therapeutic levels for purposes such as growth enhancement, prophylaxis or reduction of disease outbreaks, improvement of digestion and increase in feed conversion ratio (Donoghue, 2003; Al-Bahry *et al.*, 2006). The inhibitory effect of antibiotic growth promoters on pathogenic microflora as well as the adverse impact of their toxic metabolites have long been documented (Van Immerseel *et al.*, 2004; Cornelison *et al.*, 2006). Moreover, Saikali and Singh (2003) have enunciated concern about the overuse of antibiotics in producing food animals. Antibiotic residue in animal products and its impact on human health is of major concern. It has been reported that antibiotics are known to disrupt the ecosystem of the intestinal microflora, resulting in drug resistance (Karami *et al.*, 2006). Adopting an antibiotic-free system of production whereby natural alternatives can replace the use of dietary antibiotics is a current area of research focus.

Probiotics are potential substitutes for antibiotics in the production of food animals (Zulkifli *et al.*, 2000); they are live microorganisms that, upon their adequate administration, confer a health benefit on the host (FAO/WHO, 2001). Probiotics benefit livestock and poultry by eliminating pathogens, improving gastrointestinal barrier function, and favorably modulating the immune system (Ng *et al.*, 2009). Juven *et al*. (1991) have documented the exact role played by lactic acid bacteria (LAB) as probiotics. Similarly, Okeke *et al.* (2015) have reported the presence of LAB genera: *Lactobacillus* and *Leuconostoc* in the supernatant of fermented maize (SFM) (*Omidun*) and fermented souring (*ogi*). Furthermore, SFM has been reported by Falana *et al*. (2011) that it contains some microorganisms, such as *Lactobacillus plantarum*, that has exacted antimicrobial effect on some pathogenic microorganisms like *Escherichia coli* (Falana *et al*., 2012). However, there is a dearth of information on the use of SFM as a potential source of probiotic to enhance productivity and serve as a potential replacement for antibiotics used as growth promoters in the production of broiler chickens. Thus, the current study was carried out to investigate the effect of SFM on growth performance, livability and haematology of broiler chickens.

Materials and Methods

Ethical approval

Ethical approval was given by the Faculty Animal Research Ethics Committee, Usmanu Danfodiyo University, Sokoto. AUP No was 2019/RO-1.

Experimental design and birds' management

One hundred and fifty eight-day old Cobb 500 broiler chicks from Olam Hatcheries Limited, Kaduna State, were randomly divided into five treatment groups (A, B, C, D, and E) of 30 birds, each having three replicates. The experiment was carried out at the Poultry Unit of the Department of Theriogenology and Animal Production, Usmanu Danfodiyo University, Sokoto, and it lasted for six weeks. The birds were managed on a deep litter. A commercial feed, (Crown Flour Mill Limited), containing 22% crude protein (CP) and 2,800 kcal/kg metabolizable energy (ME), was supplied ad libitum along with water, and the pens were kept clean. The birds were vaccinated against Gumboro virus disease at weeks 1 and 3, while the *La sota* vaccine against Newcastle virus disease was administered at weeks 2 and 4 of age.

Preparation of supernatant of fermented maize (SFM) and standardization of pure isolates of LAB

The yellow maize variety was sourced from a local market within the Sokoto metropolis and was used for the preparation of SFM according to the methods of Ayorinde *et al.* (2017). The standardization of SFM was achieved by 0.5 McFarland turbidity standards (McFarland, 1907). The accuracy of the density of the McFarland standard was checked using a spectrophotometer at an absorbance reading of 0.08 to 0.1 at 630 nm.

Administration of supernatant of SFM and commercial probiotics and data collection

The birds were acclimatized from days 1 to 7, after which standardized LAB was administered in their drinking water from days 8 to 49. Birds in group A served as the negative control as they were given water without SFM, those in group B were given commercially prepared probiotics (Beta probiotics) containing *Lactobacillus* spp, *Bacillus cereus, Bacillus subtilis*, protease, lipase and *amylase* at 2.7 $\times 10^6$ CFU/ml, 6.5 $\times 10^6$ CFU/ml, 7.8 $\times 10^6$ CFU/ml, 250,000 IU, 70,000 IU and 500,000 IU respectively via drinking water, while groups C, D and E were given standardized SFM at 10, 5 and 2.5 mL/L of drinking water respectively daily from days 8 to 49 of age.

Growth performance parameters of body weight (BW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were determined weekly as described by Mountzouris *et al.* (2007). Observed mortality was recorded as the experiment progressed. The packed cell volume (PCV) was determined by the hematocrit method, using microcapillary tubes, a microhematocrit centrifuge and a reader (Coles *et al.*, 1986). The PCV result was recorded as a percentage of blood volume. The haemoglobin concentration (HbC) was determined by the cyanmethemoglobin method (Kachmar, 1970). Twenty microlitres of the blood sample were added to 5 ml of Drabkin's haemoglobin reagent in a clean test tube and allowed to react for 20 minutes. The absorbance of the mixture was read using the heamoglobin metre machine (version XF-1C), and the result displayed on the screen was obtained and recorded in tabular form in grammes per decilitre (g/dL). The red blood cell (RBC) count was obtained by the hemocytometer method (Schalm *et al.*, 1975) using an improved Neubauer counting chamber (NCC) and avian RBC diluting fluid (Natt-Hettick's solution), and the result of the 5 squares obtained was divided by 20 and then expressed in logarithm ($\times 10^{12}$/l). The total white blood cell count (WBC) was done by the hemocytometer method (Schalm *et al.*, 1975), using an improved NCC and avian white blood cell diluting fluid composed of aqueous phloxine, propylene, glycol and sodium carbamate. The WBC in the four outer large squares of the hemocytometer was counted and calculated using the formula: N/20 = WBC ($\times 10^9$/l). The differential leukocyte count (DLC) was done using the Leishman technique (Coles *et al.*, 1986). The total of 100 cells was counted by means of a DLC blood cell counter, and the Neubauer was recorded. The results of each cell type were expressed as a percentage of the total WBC count and then converted to absolute values ($\times 10^9$/l) by the following formula:

Percentage of WBC counted × total WBC/100 = Absolute Number $\times 10^9$.

Statistical analysis

All data collected were analyzed using one-way repeated measure ANOVA and were presented in Tables as means ± SEM. In all the analyses, means were significantly different at 5% ($p<0.05$), and Tukey's Test was used for all pairwise comparison.

Results

The result on growth parameters is summarized in Table 1. It was observed that supplementation of SFM in drinking water has no significant effect ($p>0.05$) on the growth and survivability of broiler chickens. However, the mean BW ranges between 994.408 ± 20.71 g/bird and 1090.013 ± 20.71 g/bird in Groups E and C, respectively. The mean haematological parameters are shown in Table 2, and it revealed a no significant difference ($p>0.05$). The results indicate variations in haematological parameters among the treatment groups.

Table 1. Growth performance and survivability of birds given supernatant of fermented maize and commercial probiotics

Group	BW (g/bird)	BWG (g/bird)	FI (g/bird)	FCR	Survivability (%)
A	1005.867 ± 20.71	266.77 ± 7.77	530.77 ± 17.54	2.01 ± 0.06	75.49
B	1034.389 ± 20.71	268 ± 7.77	582.76 ± 17.54	2.16 ± 0.06	89.77
C	1090.013 ± 20.71	290.23 ± 7.77	566.64 ± 17.54	1.93 ± 0.06	93.22
D	1037.772 ± 20.71	265.17 ± 7.77	549.96 ± 17.54	2.25 ± 0.06	82.45
E	994.408 ± 20.71	267.05 ± 7.77	566.92 ± 21.48	2.25 ± 0.08	82.37

Key: BW = Body weight; BWG = Body weight gain; FI = feed intake; FCR = Feed conversion ratio; A = Negative control; B = Positive control given commercially prepared probiotics; C = SFM at 10 ml/l; D = SFM at 5 ml/l; E = SFM at 2.5 ml/l; SEM=Standard error of mean

Table 2. Haematological parameters of birds given supernatant of fermented maize and commercial probiotics

Parameters	Groups				
	A	**B**	**C**	**D**	**E**
PCV (%)	25.22 ± 0.95	26.03 ± 0.95	26.167 ± 0.949	26.806 ± 0.949	23.861 ± 0.95
Hb (g /dL)	7.92 ± 0.28	8.22 ± 0.28	8.25 ± 0.28	8.33 ± 0.28	7.58 ± 0.28
RBC ($\times 10^6$ cells/mm^3)	2.50 ± 0.78	2.58 ± 0.78	2.83 ± 0.78	2.78 ± 0.78	2.67 ± 0.78
WBC ($\times 10^3$ cells/mm^3)	8.86 ± 0.59	8.83 ± 0.59	9.06 ± 0.59	9.53 ± 0.59	8.31 ± 0.59
Heterophil (%)	26.94 ± 1.66	28.67 ± 1.66	25.81 ± 1.66	25.06 ± 1.66	22.77 ± 1.82
Lymphocyte (%)	65.58 ± 3.08	64.33 ± 3.08	68.72 ± 3.08	70.17 ± 3.08	68.86 ± 3.08
Monocyte (%)	2.75 ± 0.38	1.39 ± 0.38	2.44 ± 0.38	2.00 ± 0.38	2.25 ± 0.38
H/L ratio	0.41 ± 0.54	0.45 ± 0.54	0.38 ± 0.54	0.36 ± 0.54	0.33 ± 0.59

Key: RBC = Red Blood Cells; WBC = White Blood Cells; Hb = Hemoglobin; PCV = Packed Cell Volume; H/L = Heterophils to Lymphocyte ratio; A = Negative control; B = Positive control given commercially prepared probiotics; C = SFM at 10 ml/l; D = SFM at 5 ml/l; E = SFM at 2.5 ml/l; SEM=Standard error of mean

Discussion

This study investigated the potential of supernatant of fermented maize (SFM) (*Omidun*) as a probiotic source on growth performance, survival rate and haematology of broiler chicken. The SFM has been traditionally found to be of medicinal importance (Falana *et al.*, 2016). This may be attributed to the fact that it contains some probiotics in the form of lactic acid bacteria. The SFM has been reported by Falana *et al.* (2011) to contain some microorganisms including *L. plantarum.* They also reported that these microorganisms have antimicrobial efficacy against some pathogenic microorganisms including *Escherichia coli* (Falana *et al.*, 2012).

The beneficial effects of supplementation of lactic acid bacteria (LAB) on growth broiler's performance by improving digestive function, increasing the bioavailability of dietary micronutrients, modulating intestinal microflora, enhancing immuno-modulation and better the health of the broiler (Gao *et al.*, 2008; Higgins *et al.*, 2008). It was observed that, there was a progressive increase in body weight throughout the duration of the treatment, with the 10 ml/L of SFM (group C) recorded the highest body weight throughout the duration of the research wok. This agrees with the work of Brzoska and Stecka (2007) and Brzoska *et al*. (2012) in which the probiotic bacteria increased chickens' body weight. The group with highest dose of SFM (group C) recorded the highest weight gain, although not significant, throughout the duration performance was supported by the works of Mountzouris *et al.* (2007). The LAB may enhance digestion by increasing the surface area and length of intestinal villi (Banasaz *et al.*, 2002). The gut microbiota affects the digestion, absorption and metabolism of dietary carbohydrates, protein, lipids, minerals and the synthesis of vitamins (Jin *et al.*, 1997).

In the present study, the treatment group with the 10 m/L of standardized SFM maintained steady and higher growth rate as compared to the controls and other treated groups though not significantly different but this increased in growth might have significant economic impact. It has been suggested that LAB can stimulate of the research. This agrees with the work of Mountzouris *et al*. (2007) in which probiotic treatment performed well in terms of overall body weight gain and feed conversion ratio.

The hematocrit values (HCT) observed in the present study among the group treated with the probiotic is at variance with the study done by Cetin *et al*. (2005), who observed that the probiotic supplementation caused statistically significant increase in the erythrocyte count, haemoglobin concentration and hematocrit values of turkeys. However, this outcome, is in agreement with the work of Djouvinov *et al*. (2005), who equally reported that the probiotic supplementation did not affect the blood constituents comprising haemoglobin concentrations. The differences may be attributed to type and strain of birds used in the two studies. Alternatively, the observed differences may be attributed to the types and number of species of bacteria present in probiotics used. There was no statistically significant difference in the heterophil to lymphocyte ratio. This result is in consonance with the work of Sarinee *et al.* (2008) who observed that when the probiotic was added to the drinking water of male Cobb broiler chickens, there was no significant effect in the H/L ratio at 28 and 42 days old compared to the control group.

On the effect of treatment on mortality, the result showed that lowest mortality rate (6.78%) was recorded in group having highest dosage of *Omidun* (Group C) followed by 10.23%, 17.55%,17.63% and 25.51% for the treated group B, D, E and control (A) respectively. This agrees with the work of Timmerman *et al.* (2006) and Bostami *et al.* (2015) and in which probiotic organisms reduced mortality because of their synergistic and biotherapeutic effects which remarkably decrease mortality as observed in broiler after probiotic administration. This was also in agreement with the works of Wang *et al.* (2018) who inoculated hatched chicks with 1×10^9 CFU of *L. plantarum* LTC-113 strain, which provided anti-*Salmonella typhimurium* protection by limiting the gut colonization and stabilizing the expression of tight junction genes in intestinal epithelial cells among treated chickens making them more resistant to the infection. Hence, combating mortality that would have risen via infectious agents. An important function of probiotic bacteria is to provide defense to the host gastrointestinal tract from pathogens (Gill and Cross, 2002; Zhang and Kim, 2014).

Conclusion

It can be concluded that the supplementation of supernatant of fermented maize (SFM) as probiotics, could have positive effects on the productive performance of broiler thereby improving the haematological parameters.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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