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Enhancing Broiler Chicken Performance, Gut Microbiota, and Carcass Traits Through Prebiotics (Mannan-Oligosaccharides) and Probiotics (*Saccharomyces Cerevisiae*)

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ABSTRACT

This study was conducted to investigate the effect of prebiotics (MOS) and probiotics (yeast) on the performance, caecal microbiota, and carcass traits of broiler chicken as an alternative to antibiotics. Accordingly, 300-day-old Cobb-500 broiler chicks were randomly assigned to five treatment groups, with three replicates, each containing 20 chicks. Dietary treatments were formulated as follows: (i) control; (ii) antibiotic (0.25 g/kg feed); (iii) MOS (0.5 g/kg feed) + yeast (1.5 g/kg); (iv) MOS (1 g/kg feed) + yeast (2 g/kg); (v) MOS (1.5 g/kg feed) + yeast (2.5 g/kg). Results showed that body weight gain, feed intake, and feed conversion ratio were significantly influenced ($P < 0.05$) by MOS and yeast in all the treatment groups compared to the antibiotics and control. Similarly, supplementation with MOS and yeast showed a positive effect ($P < 0.05$) on the breast, thigh, wing, back, and gizzard weight but did not significantly ($P > 0.05$) affect the liver weight. All treatments were found to have significantly lower ($P < 0.05$) *E. coli* and Salmonella populations than the control. Thus, it may be concluded that MOS (1 g/kg feed) and yeast (2 g/kg feed) may be incorporated into broiler diets for better performance as an alternative to antibiotics.

KEYWORDS: Antibiotics, Broiler chickens, Caecal microflora,Carcass traits

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INTRODUCTION

The rapid growth of the poultry industry is closely linked to consumers increasing demand for a healthier diet, where meat plays an essential role. The establishment of new broiler farms is increasing day by day to meet the global demand for meat, and antibiotics have been used for a long time as a growth promoter. The residual effect of antibiotics on human health is increasing at an alarming rate, and therefore, it's time to ban the use of antibiotics in broiler feed. Moreover, concerns about antimicrobial resistance in bacteria from poultry production are increasing due to the existence of antimicrobial residues in meat (Reigh and Toldra, 2008) and eggs (Goetting et al., 2011), and for this reason alternatives are currently being suggested and pursued, specifically, targeting probiotics and prebiotics for use in the poultry industry (Zhang et al., 2012). A popular alternative to the use of antibiotics has been the use of probiotics and prebiotics, which have been used in poultry for competitive or exclusion of bacterial pathogens (Barrow, 1992). The positive effects of probiotics on broilers can result either from a direct nutritional effect or a health effect of the probiotics (Shareef and Al-Dabbagh, 2009). Some clinical studies have concentrated on general outcomes, often overlooking the nuanced responses that the combined effects of prebiotics and probiotics may elicit. Moreover, there is a pressing need for more comprehensive investigations into the long-term impact of prebiotic and probiotic supplementation on broiler health and productivity. Research addressing optimal dosage, the timing of administration, and potential synergistic effects resulting from combining specific prebiotics with distinct probiotic strains. Therefore, the present study aims to investigate the dietary effects of prebiotics and probiotics on the performance, caecal microflora, and carcass traits of broiler chicken as an alternative to antibiotics.

MATERIALS AND METHODS

Ethical approval

This experiment was carried out in accordance with the National Regulations on Animal Welfare and the guidelines set by the Institutional Animal Ethics Committee.

Place of work

This study was conducted at the poultry farm of the Department of Animal Nutrition, Genetics, and Breeding at Sher-e-Bangla Agricultural University, located in Sher-e-Bangla Nagar, Dhaka-1207.

Experimental design and birds

A total of 300 day-old Cobb-500 commercial broiler chicks of uniform body weight (average 42.2g) were used in this study. The birds were divided randomly into five treatment groups with three replicates each and 20 birds in each replicate with equal numbers of males and females. Among the five dietary treatment groups, one group was fed a basal diet and served as the control, while the other groups were fed the same diet supplemented with MOS (0.5 g/kg feed) + yeast (1.5 g/kg feed), MOS (1 g/kg feed) + yeast (2 g/kg feed), MOS (1.5 g/kg feed) + yeast (2.5 g/kg feed) and antibiotic (0.25 g/kg feed). The experiment was arranged following completely randomized design (CRD) principles.

Experimental diet

According to the basal diet of broiler chicks recommended by the US National Research Council, a maize-soybean basal diet was prepared. A starter diet was provided for 1-14 days, and a grower diet for 15-28 days. The nutrient requirements were satisfied to the requirements of Cobb-500 commercial broilers. The compositions and nutrient levels of the basal diet are mentioned in Table 1.

Table 1. The ingredients and chemical composition of the basal diet

Item	Starter phase 1 to 14 (day)	Grower phase 15 to 28 (day)
Maize, 7.4% CP	54.7	55.7
Soybean meal, 44.5 % CP	37.9	36.1
Soybean oil (%)	2.30	3.28
Oyster shell (%)	1.58	1.50
Sodium bicarbonate (%)	0.19	0.17
Dicalcium phosphate (%)	2.02	1.90
Salt (NaCl) (%)	0.20	0.23
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
DL- Methionine (%)	0.34	0.32
L- Lysine HCL (%)	0.20	0.16
L-Threonine (%)	0.08	0.06
	Chemical composition	
ME (kcal/kg)	2900.00	3000.00
CP (%)	22.1	20.7
Methionine (%)	0.65	0.90
Lysine (%)	1.26	1.23
Methionine + Cysteine (%)	0.90	0.82
Calcium (%)	0.92	0.84
Available phosphorus (%)	0.41	0.38

*Supplied per kilogram of diet: Vitamin A, 10,000 IU; vitamin D_3, 2,000 IU; vitamin E, 10 mg; vitamin K, 20 mg; vitamin B_1, 2 mg; vitamin B_2, 10 mg; vitamin B_3, 15 mg; vitamin B_6, 300 mg; vitamin B_5, 10 mg; vitamin B_8 5mg;vitamin B_9, 250 mg. ** Supplied per kilogram of diet: Manganese 500 mg; iron 250 mg, iodine 10 mg, zinc 600 mg; copper 100 mg, selenium 1 mg and cobalt 1 mg.

Management practices

Before beginning the research trial, the experimental shed, feeder, drinker, and other equipment were washed and disinfected. The birds received consistent care and management throughout the experimental period. Upon receiving day-old chicks on the farm, they were weighed and then randomly distributed to electrically heated brooders, where they were reared for seven days. Then, the birds were distributed to each pen according to the experimental design. The birds were exposed to light from a fluorescent bulb for 23 hours, with a one-hour period of darkness maintained throughout the brooding period. Subsequently, the lighting schedule was adjusted according to the standard level. The birds were housed in 3ft by 2ft floor pens using fresh rice husk litter with a depth of 3 cm, and they were provided with a 24-hour lighting plan. An automatic digital thermo-hygrometer maintained the optimum temperature and humidity levels throughout the experiment, adjusting according to the age of the birds. One round tube feeder and one round drinker were provided in each pen. According to the Cobb-500 feeding manual, feed was supplied four times daily and water two times daily. Left-over feeds were registered to measure actual feed consumption. Fresh and clean drinking water was provided *ad libitum*, and feeders and drinkers were subjected to cleaning when required. The scale of the feeder and drinker was adjusted according to the age of the birds. The feed and body weight of birds were weighted on days 1, 14, and 28 to calculate feed intake (FI), body weight gain (BWG), and feed conversion rate (FCR).

Vaccination program

Vaccination was applied to the experimental birds according to the vaccination schedule. The cool vaccine chain was exclusively maintained until vaccination. The birds were vaccinated on a proper schedule against New Castle disease, infectious bronchitis and infectious bursal disease.

Growth performance and characteristics of carcass

Body weight gains (BWG) of broiler chickens were measured weekly using a digital electronic weighing scale in each replication. Feed intake (FI) and feed conversion ratio (FCR) were calculated and analyzed throughout each week. Towards the end of the 28-day trial period, nine birds from every treatment (three birds for each replicate) were slaughtered after 12 hours of fasting, with *ad libitum* drinking water, using the halal method, severing the jugular vein, carotid artery, and trachea through a single incision with a sharp knife, allowing them to completely bleed out for at least 2 minutes. Subsequently, the carcass was eviscerated, dissected and the cut-up yield (breast, thigh, drumstick, wing, back, liver, and gizzard) was determined.

Collection of sample and microbiological analysis

Both caecal pouches were collected from each bird and immediately placed in a portable freezer at -20!. They were then transported to the laboratory and stored at -80°C for microbial enumeration. 1 mL of the homogenized suspension was transferred into 9 mL of anaerobic broth and serially diluted from 10^{-1} to 10^{-6} in phosphate buffer solution from which 100 µL was plated on agar plates. To isolate Salmonella and *Escherichia coli* bacteria, the diluted samples were seeded on *Salmonella Shigella* (SS) agar and Eosin Methylene Blue (EMB) agar, respectively. The plates were then incubated for 48 hours at 37°C. *Escherichia coli* and Salmonella colonies were counted immediately upon removal from the incubator. Results were expressed as log10 colony-forming units per gram of cecum digesta (Hashemi et al.,2012).

Statistical analysis

The data were analyzed using the General Linear Model procedure (IBM SPSS software version 20).Significant differences among treatments were tested using one-way analysis of variance (ANOVA) followed by Duncan multiple comparison test. The level of statistical significance was set at $P < 0.05$ with the standard error of the means.

RESULTS AND DISCUSSION

Growth performance

The results, determined based on performance parameters such as live weight, body weight gain, feed intake, and feed conversion ratio are summarized in Table 2. Birds that received prebiotics (MOS) and probiotics (yeast) consumed significantly less feed and gained maximum body weight. Consequently, the feed conversion ratio was significantly better (PÂ0.05) when comparing the experimental birds treated with prebiotics (MOS) and probiotics (yeast) to the control group from the first week until the end of the experiment. The average live weight, body weight gain, feed intake and feed conversion ratio varied significantly (PÂ0.05) across the dietary supplemented groups, with the better result found in the T4 group (yeast 2g/kg and MOS 1g/kg feed) followed by T5, T3, T2 and T1 groups.

In this study, the results indicate that the inclusion of prebiotic (MOS) and probiotic (yeast) supplementation has positive effects on broiler growth performance. These findings align with the studies of Gao et al. (2017) and Ding et al. (2019), who reported that including yeast culture or yeast-derived products in broiler diets could improve body weight gain (BWG). In contrast, Munyaka et al. (2012) found that probiotics (*Saccharomyces cerevisiae*) had no effect on growth performance. Fernandes et al. (2014) reported that MOS supplementation during the first week of age led to greater feed intake compared to the control group. Similarly, studies by Abdel-wahid et al. (2017), Shahir et al. (2014) and Moila et al. (2020) all found that the MOS-supplemented group showed better BWG than the control group. However, Attia et al. (2014) reported that no significant difference was observed in BWG during 1-21 days of age between the MOS-supplemented and positive control groups.

Table 2.Effects of prebiotics (MOS) and probiotics (yeast) on average live weight (LW), body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) in broiler chicken

Treatment	LW (g/bird)	BWG (g/bird)	FI (g/bird)	FCR (g/bird)
T1	$1634^{d}\pm2.33$	$1592^{d}\pm2.33$	$2275^{d}\pm0.57$	$1.42^{a}\pm0.01$
T2	$1692^{c}\pm1.45$	$1650^{c}\pm1.45$	$2269^{b}\pm0.56$	$1.37^{b}\pm0.01$
T3	$1717^{b}\pm1.45$	$1675^{b}\pm1.45$	$2273^{a}\pm0.57$	$1.35^{c}\pm0.01$
T4	$1728^{a}\pm0.88$	$1687^{a}\pm0.88$	$2231^{e}\pm0.55$	$1.32^{e}\pm0.01$
T5	$1720^{b}\pm0.57$	$1678^{b}\pm0.57$	$2253^{c}\pm1.52$	$1.34^{d}\pm0.01$

Here, T1 = (control), T2 = (antibiotic 0.25 gm/kg feed), T3 = (yeast 1.5 g/kg and MOS 0.5 g/kg feed), T4 = (yeast 2 g/kg and MOS 1 g/kg feed), T5 = (yeast 2.5 g/kg and MOS 1.5 g/kg feed), and values indicate mean ± standard error. Mean with different superscripts are significantly different ($P<0.05$).

Feed intake

Current research illustrates that the addition of prebiotics (MOS) and probiotics (yeast) significantly reduced feed intake and increased maximum body weight in the dietary treatment group compared to the control group. This may be attributed to the birds absorbing nutrients more effectively, leading to reduced feed requirements to meet their nutritional needs. Sousa et al. (2019) described that when broilers were fed with 6% yeast (SC), no significant differences were detected in feed intake (FI) in broilers. In contrast, Paryad and Mahmoudi (2008) found that the inclusion of 1.5% S. cerevisiae yeast in broiler rations improved feed intake. Ijiet al. (2001) revealed that the addition of MOS to the diet enhanced the feed intake of birds compared to the control group. However, Abdel-wahidet al. (2017), Kocet al. (2010), and Al-Sultan et al. (2016) observed that MOS supplementation had no effect on feed intake (FI).

Feed conversion ratio (FCR)

The present study illustrates that the supplementation of prebiotics (MOS) and probiotics (yeast) has a positive effect on FCR. Bozkurt et al. (2008) reported similar findings, observing better FCR in the MOS-added group compared to all other groups (negative control, positive control with AGP, and dextran oligosaccharide). Similarly, Santin et al. (2001) also aligned with the present study, finding better FCR in the MOS-supplemented group. However, the results in the study by Abdel-wahidet al. (2017) contrasted with the present findings, as they reported no significant differences in FCR due to MOS supplementation. Paryad and Mahmoudi (2008) found that the inclusion of 1.5% *S. cerevisiae* yeast in broiler rations improved FCR. On the other hand, the results presented in the study by Koc et al. (2010) contrasted with the present findings, as they reported that FCR tended to decrease with *Saccharomyces cerevisiae* and MOS supplementation.

Characteristics of carcass

The effect of prebiotics (MOS) and probiotics (yeast) on carcass characteristics is presented in Table 3. No significant differences (P > 0.05) were found in liver and drumstick among the treatment groups, while significantly better breast, thigh, wing, back, and gizzard weights (% of live weight) were recorded in the T4 group. The T3 group showed statistically comparable results, and the T5 group also exhibited significantly better result compared to the control and antibiotic-supplemented group. Similar to the results of the present study, Toghyani and Tabeidian, (2011) reported significantly higher cut-up part yields in chickens fed diets containing MOS. Therefore, based on the results of the present study, it can be assumed that the application of prebiotics and probiotics has a positive effect on carcass weight. However, in contrast to the present study, Rehman et al. (2020) and Ricke (2018) reported no significant differences in breast and thigh weights after the dietary inclusion of MOS. On the other hand, the results presented in the study by Koc et al. (2010) and Sinha et al. (2017) contrast with the present study, as they reported that supplementation of *Saccharomyces cerevisiae* with MOS showed no significant differences in organ weights, except for gizzard and duodenum weights.

Table 3. Effects of prebiotics (MOS) and probiotics (yeast) on organ relative weight of broiler chicken in 28 days

Treatment	Organ relative weight (% of live body weight)						
	Breast	Thigh	Drumstick	Wing	Back	Liver	Gizzard
T1	$27.1^{d}\pm0.22$	$10.1^{c}\pm0.54$	$8.86^{b}\pm0.68$	$5.02^{c}\pm0.08$	$11.7^{c}\pm0.50$	$3.03^{a}\pm0.19$	$1.67^{b}\pm0.18$
T2	$28.9^{cd}\pm0.80$	$11.2^{bc}\pm0.53$	$10.5^{a}\pm0.54$	$5.28^{bc}\pm0.30$	$12.3^{c}\pm0.56$	$3.13^{a}\pm0.15$	$1.72^{ab}\pm0.01$
T3	$32.1^{ab}\pm0.48$	$12.1^{ab}\pm0.33$	$10.9^{a}\pm0.35$	$5.68^{ab}\pm0.05$	$13.7^{b}\pm0.21$	$3.29^{a}\pm0.15$	$1.86^{ab}\pm0.13$
T4	$33.9^{a}\pm1.45$	$12.9^{a}\pm0.47$	$11.7^{a}\pm0.25$	$5.97^{a}\pm0.12$	$15.3^{a}\pm0.38$	$3.38^{a}\pm0.07$	$2.01^{ab}\pm0.20$
T5	$30.9^{bc}\pm0.66$	$11.6^{abc}\pm0.45$	$10.9^{a}\pm0.05$	$5.56^{ab}\pm0.04$	$12.7^{bc}\pm0.33$	$3.16^{a}\pm0.45$	$2.18^{a}\pm0.08$

Here, T1 = (control), T2 = (antibiotic 0.25 gm/kg feed), T3 = (yeast 1.5 g/kg and MOS 0.5 g/kg feed), T4 = (yeast 2 g/kg and MOS 1 g/kg feed), T5 = (yeast 2.5 g/kg and MOS 1.5 g/kg feed), and values indicate mean ± standard error. Mean with different superscripts are significantly different (P<0.05) and mean within same superscripts don't differ (P>0.05)significantly

Microbial load

The results of microbial load in the caeca, influenced by prebiotics (MOS) and probiotics (yeast) supplementation, are presented in Table 4. The study revealed that the control group (T1) had the highest populations of Salmonella and *E. coli.* In contrast, the T4 group exhibited the lowest numbers of Salmonella and *E. coli* populations. The results of the present study showed that the supplementation of *Saccharomyces cerevisiae* with MOS reduced Salmonella and *E. coli* bacteria. The results of Koc et al. (2010) were similar to those of this study; they observed that supplementation of *Saccharomyces cerevisiae* alone or in combination with MOS significantly reduced caecal microflora. Similarly, Sinha et al. (2019) and Newman (1994) revealed that supplementation of MOS in the diet of broiler chicken showed significant (P<0.05) lowering in cfu count of pathogenic bacteria as *E. coli* and *Salmonella spp.* as compared to the control group. In the present study, *Saccharomyces cerevisiae* with MOS had more beneficial effects compared to the other treatments, especially the control groups.

Table 4. Effects of prebiotics (MOS) and probiotics (yeast) on caecal bacterial population (log10 CFU/g) in broiler chicken

Treatment	*Escherichia coli*	*Salmonella*
	Mean ± SE	Mean ± SE
T1	5.93^{a}±0.01	5.95^{a}±0.01
T2	5.84^{b}±0.02	5.83^{b}±0.00
T3	5.84^{b}±0.02	5.82^{b}±0.01
T4	5.63^{c}±0.02	5.75^{c}±0.01
T5	5.82^{b}±0.01	5.82^{b}±0.01

Here, T1 = (control), T2 = (antibiotic 0.25 gm/kg feed), T3 = (yeast 1.5 g/kg and MOS 0.5 g/kg feed), T4 = (yeast 2 g/kg and MOS 1 g/kg feed), T5 = (yeast 2.5 g/kg and MOS 1.5 g/kg feed), and values indicate mean ± standard error. Mean with different superscripts are significantly different (P<0.05).

CONCLUSION

The results extracted from the following research indicate that birds fed diets containing yeast and MOS exhibited improvements in body weight gain and better productive performance compared to the control and antibiotic groups. In conclusion, yeast and MOS can be used in place of AGPs and can play a role in minimizing the irrational use of antibiotics in poultry feed but further research is needed to verify the efficacy of yeast and MOS as alternatives to antibiotics in broiler production.

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