Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity

A. Rehman,* M. Arif,* N. Sajjad,* M. Q. Al-Ghadi,† M. Alagawany,‡,† M. E. Abd El-Hack,‡ A. R. Alhimaidi,† S. S. Elnesr,‡ B. O. Almutairi,† R. A. Amran,† E. O. S. Hussein,# and A. A. Swelum,‡,#,†

*Department of Animal Sciences, College of Agriculture, University of Sargodha, Sargodha 40100, Pakistan; †Department of Zoology, College of Sciences, King Saud University, Riyadh, Saudi Arabia; ‡Department of Poultry, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt; #Department of Poultry Production, Faculty of Agriculture, Fayoum University, Fayoum, Egypt; and Department of Theriogenology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

ABSTRACT This experiment was carried out to evaluate the effects of dietary addition of probiotics (Protexin) and prebiotics (active MOS, mannan oligosaccharides) on growth performance, carcasses, and antibody titer in broilers. A total number of 360-day-old Ross broiler chicks were randomly divided into 9 groups in a 3 x 3 factorial arrangement. Nine broiler starter (0–21 d) and finisher (21–35 d) diets were formulated by using 3 levels of probiotics (0, 1, and 2 g/kg of feed) and 3 levels of MOS (0, 1, and 1.5 g/kg of feed) and were randomly allotted to 9 groups. Feed intake was not affected by interaction of treatments during all phases (P > 0.05). Feed intake was improved due to the main effect of probiotic (P < 0.0001) or MOS (P = 0.005). No interaction (P > 0.05) was observed for weight gain in the starter, finisher, and overall phases. While, during the starter and finisher phases, weight gain was increased by probiotics (P = 0.028 or 0.04, respectively). Dietary supplementation of MOS improved weight gain (P = 0.01) and feed conversion ratio (FCR) (P = 0.03) during the overall period, but during starter and finisher periods, weight gain and FCR were not affected by prebiotics. Apart from dressing percentage, no interaction or individual effect of probiotics and prebiotics was observed for carcass, breast, thigh, heart, liver, and gizzard weight. Antibody titer for infectious bursal disease (IBD) was improved (P = 0.026) by the interaction effect between probiotics and prebiotics, when compared with the control group. Antibody titer against Newcastle disease (ND) was not affected by probiotics or prebiotics or their interactions (P > 0.05). It could be concluded that supplementation of prebiotics or probiotics can improve the growth performance of broilers. It may also be helpful in improving the antibody titer against IBD in broilers fed antibiotic-free diets.

Key words: broilers, growth, immunity, prebiotics, probiotic

INTRODUCTION Probiotics are defined as “live microorganisms which when administered in adequate amount confer health benefits to the host” (FAO/WHO, 2002). Probiotics enhance immunity, health, and growth in all ages and class of poultry, improving a healthy balance of bacteria in the gastrointestinal tract, promoting the gut integrity and maturation, boosting the immune response and preventing inflammation, improve feed intake and digestion by increasing the activity of digestive enzyme and decreasing activity of bacterial enzyme as well as decreasing ammonia production, neutralize enterotoxins, and stimulate immune function (Kabir, 2009; Alagawany et al., 2016, 2018; Soomro et al., 2019). Probiotic species are Lactobacillus bulgaricus, Lactobacillus plantarum, Streptococcus thermophilus, Bifidobacterium bifidum, Aspergillus oryzae, etc. (Khaksefidi and Rahimi, 2005). They do not leave residues in animal products (meat, milk, and egg) and improve animal’s health and performance (Patterson and Burkholder, 2003). Probiotics modify the intestinal ecosystem by supplying digestion enzymes, reducing pH,
and increasing the activity of enzymes in the gastrointestinal tract (Kabir, 2009; Abd El-Hack et al., 2020). Probiotics greatly affect the intestinal microbiota. They work against salmonella to prevent birds from infection and increasing the activity of enzymes in the gastrointestinal tract (Kabir, 2009; Abd El-Hack et al., 2020). Probiotics modulate intestinal microbiota and reduce the pathogen, improve the immunit sensory properties of broiler meat (Pelciano et al., 2005), and promote reduce the pathogen, improve the immunit sensory properties of broiler meat (Pelciano et al., 2005), and promote the quality of microbiological meat (Kabir, 2009). Probiotics supplementation has a significant effect on carcass yield, live weight gain, immune response, and prominent cut up meat parts (Soomro et al., 2019). However, colonization of probiotics in the gut depends on many factors, including availability of fermentation substrate (prebiotics), the specificity of the strain relative to the host dose and frequency of supplementation, age, health, genetics, and nutritional status of the host, intestinal pH, and stress (Bomba et al., 2002).

The prebiotics are necessary for better survival of probiotics in the gut. Probiotics can well dwell in the digestive system with help of prebiotics as with this they can well tolerate anaerobic environment, for example, low oxygen, low pH, and temperature. The prebiotics are used as substrates for survival and multiplication of probiotics in a lower gut region that act as symbiotic (Hanamanta et al., 2011). Prebiotics have been shown to be promising in controlling pathogens such as Escherichia coli and Salmonella and stimulate the growth of Lactobacilli and Bifidobacteria. Commonly used prebiotics are mannan oligosaccharides (MOS). Mannan oligosaccharides have been derived from the outer cell wall of Saccharomyces cerevisiae. They are outer layer of yeast cell walls, including glucan 30%, mannan 30%, and protein 12.5%. The protein is high in serine, aspartic acid, glutamic acid, and methionine (Song and Li, 2001). The addition of MOS in broiler diets may have a positive effect on growth performance (Rosen, 2006). Mannan oligosaccharides are special when compared with other oligosaccharides because of their mode of action to influence microbial populations in the GIT. Mannan oligosaccharides contain a high-affinity ligand for bacteria and provide a competitive binding site. So pathogens attach to the MOS instead of the intestinal wall and move through the intestine without colonization (Benites et al., 2008). Mannan oligosaccharides increased lactobacilli and bifidobacteria populations in the ceca (Baurhoo et al., 2007) and serum concentration of IgA (Kim et al., 2009). Some previous studies investigated impacts of probiotics and prebiotics for poultry, but studies on the use of protexin with MOS are very rare. It is hypothesized that the combination between probiotic and prebiotic exhibits the powerful influence of each addition that appeared in the alone form. Thus, the present study was planned to evaluate the effect of protexin, MOS, and their combinations on broiler performance, antibody titer, and carcass characteristics.

**MATERIALS AND METHODS**

This study was conducted to evaluate the effects of dietary protexin, MOS, and their combinations on growth performance and blood biochemistry in broilers at Poultry Research Center, College of Agriculture, University of Sargodha, Sargodha. All experimental procedures of the above-mentioned study were performed according to the Local Experimental Animal Care Committee and approved by the ethics of the institutional committee of College of Agriculture, University of Sargodha, Sargodha (SARU-0021-2019).

**Birds, Design, and Experimental Diets**

Research was conducted at University College of Agriculture, University of Sargodha, and Sargodha. Three hundred sixty-day-old Ross broiler chicks (40 ± 0.05) were randomly divided into 9 groups (4 replicates per treatment and 10 birds per replicates) under completely randomized design in a 3 × 3 factorial arrangement. Nine broiler starter and finisher isonitrogenous and isocaloric diets were formulated by using 3 levels of probiotics (i.e., 0, 1, and 2 g/kg of feed) and 3 levels of prebiotics (0, 1, 1.5 g MOS/kg of feed) and were randomly allotted to 9 groups. The commercial probiotic and prebiotics were used in accordance with the manufacturer instructions. Starter diet was fed from day 1 to 21 and finisher ration was offered from 22nd to 35th day of experiment (Table 1). Birds were free-ranging in suitable pens. The ration was fed at ad libitum basis and water was made available round the clock. Manual feeding and drinking was carried out with full lighting at day 1 to 3, 18 h light

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**Table 1. Composition of experimental diets.**

| Ingredients (kg) | Starter | Finisher |
|------------------|---------|----------|
| Corn             | 25.00   | 52.65    |
| Rice tips        | 34.18   | 20.09    |
| Canola meal 37%  | 7.7     | 0        |
| Soybean meal 46% | 27.05   | 22.9     |
| Guar meal        | 1.0     | 0        |
| Lime stone       | 1.32    | 1.2      |
| MCP              | 0.84    | 0.60     |
| Sodium bicarbonate | 0.52    | 0.47     |
| Vegetable oil    | 0.71    | 0.47     |
| DL-Methionine    | 0.24    | 0.21     |
| Lysine sulfate 70% | 0.33  | 0.28     |
| Threonine        | 0.08    | 0.09     |
| Premix¹         | 1.0     | 1.0      |
| Total            | 100     | 100      |
| Nutrients %      |         |          |
| ME (kcal/kg)     | 2,930   | 3,090    |
| Dig. Lys         | 1.14    | 0.92     |
| Dig. Met + Cys   | 0.85    | 0.71     |
| CF               | 2.52    | 1.83     |
| Ash              | 5.6     | 4.61     |
| Ca               | 0.90    | 0.76     |
| P Total          | 0.70    | 0.60     |
| P Available      | 0.45    | 0.38     |
| Na               | 0.18    | 0.19     |
| Cl               | 0.05    | 0.05     |
| K                | 0.72    | 0.63     |

¹Provides per kg of diet: Vitamin A, 12,000 I.U; Vitamin D₃, 5000 I.U; Vitamin E, 130.0 mg; Vitamin K₃, 3.005 mg; Vitamin B₁ (thiamin), 3.0 mg; Vitamin B₂ (riboflavin), 8.0 mg; Vitamin B₆, 4.950 mg; Vitamin B₁₂, 17.0 mg; Niacin, 60.0 mg; D-Biotin, 200.0 mg; Calcium D-pantothenate, 18.333 mg; Folic acid, 2.083 mg; manganese, 100.0 mg; iron, 80.0 mg; zinc, 80.0 mg; copper, 8.0 mg; iodine, 2.0 mg; cobalt, 500.0 mg; and selenium, 150.0 mg.
at day 4 to 7, and then was maintained 23 h till the end of trial. Biosecurity measures were adopted. Phenyl was spread at entrance and at exit passage during the whole trial on a daily basis. Protexin is a multistrain probiotic, contains L. plantarum, Lactobacillus rhamnosus, Enterococcus faecium, Candida pintelesii, Bifidobacterium bifidum, and A. oryzae in isolated forms (not less than $6 \times 10^7$ cfu/g) (Australian Co., Ltd.). Probiotics and prebiotics were purchased from Global Engage Sdn Bhd, level 33, Ilham Tower, No. 8 Jalan Binjai, 50450 Kuala Lumpur, Malaysia.

### Growth Performance

Daily feed intake per replicate was recorded to compute feed intake per week. Chick's body weight was recorded at the time of arrival and after every week of age by using an electrical weighing balance. Values of feed intake and weight gain were used to calculate feed conversion ratio (FCR).

### Antibody Titer Against Newcastle and Infectious Bursal Disease

For antibody analyses, at 30th day of age, 1 mL blood was taken by wing vein puncture of 2 birds per replicate. Blood samples were collected in nonheparinized tubes and tubes were kept for 2 h at 45°C and serum was taken for analyzing antibody titers against Newcastle disease (ND) by hemagglutination inhibition test and infectious bursal disease (IBD) was determined by using an enzyme-linked immune sorbent assay kit according to Beard et al. (1975).

### Carcass Yield

At the end of the experiment, 2 chickens from each replicate were randomly selected and slaughtered to study carcass characteristics and organ weight of the broiler birds. Hot carcass, heart, liver, breast, thigh, and abdominal fat were weighed to the nearest 0.01 g on a

| Items | MOS0 | MOS1 | MOS1.5 | MOS0 | MOS1 | MOS1.5 | MOS0 | MOS1 | MOS1.5 | SEM | Sig |
|-------|------|------|--------|------|------|--------|------|------|--------|-----|-----|
| Feed intake (g) | | | | | | | | | | | |
| 0–21 d | 770.3 | 819.7 | 799.7 | 825.6 | 878.2 | 900.8 | 833.5 | 825.5 | 883.3 | 17.6 | 0.173 |
| 22–35 d | 1,255 | 1,398 | 1,411 | 1,422 | 1,397 | 1,367 | 1,396 | 1,344 | 1,425 | 52.01 | 0.177 |
| 0–35 d | 2,109 | 2,217 | 2,210 | 2,248 | 2,276 | 2,268 | 2,268 | 2,199 | 2,169 | 2,308 | 51.4 | 0.47 |
| Weight gain (g) | | | | | | | | | | | |
| 0–21 d | 468.1 | 505.3 | 488.5 | 516.7 | 526.7 | 532.3 | 493.6 | 508.6 | 542.5 | 16.8 | 0.601 |
| 22–35 d | 804.5 | 893.3 | 846.8 | 932.2 | 936.3 | 936.9 | 957.3 | 892.9 | 948.8 | 43.71 | 0.94 |
| 0–35 d | 1,273 | 1,429 | 1,337 | 1,439 | 1,451 | 1,443 | 1,450 | 1,427 | 1,492 | 41.7 | 0.23 |
| Feed conversion ratio (g feed/g gain) | | | | | | | | | | | |
| 0–21 d | 1.65 | 1.63 | 1.64 | 1.61 | 1.67 | 1.70 | 1.70 | 1.63 | 1.63 | 0.529 |
| 22–35 d | 1.71 | 1.58 | 1.68 | 1.52 | 1.51 | 1.42 | 1.51 | 1.58 | 1.52 | 0.085 | 0.78 |
| 0–35 d | 1.68 | 1.57 | 1.68 | 1.55 | 1.58 | 1.49 | 1.55 | 1.63 | 1.56 | 0.04 | 0.13 |

Probiotic 0, 1, and 2 indicate inclusion of Protexin at the rate of 0, 1, and 2 g/kg of feed. MOS 0, 1, and 1.5 indicate inclusion of Active-MOS (mannan oligosaccharide) at the rate of 0, 1, and 1.5 g/kg of feed. Abbreviation: Sig, significant.
The data collected during this experiment was statistically analyzing under completely randomized design in $3 \times 3$ factorial arrangements (Steel et al., 1996). All data were tested for distribution normality and homogeneity of variance. A $3 \times 3$ factorial design was used to analyze data of performance as a response to 3 levels of probiotics (i.e., 0, 50, and 100 g/50 kg of feed) supplemented with prebiotics MOS (0, 50, 75 g/50 kg of feed). Differences among means were detected using Tukey's test ($P < 0.05$). The model used was:

$$Y_{ij} = \mu + D_i + A_j + DA_{ij} + e_{ij}$$

Where $Y_{ij}$ is an observation, $\mu$ is the overall mean, $D_i$ is fixed effect of probiotics, $A_j$ is fixed effect of prebiotics, $DA_{ij}$ is fixed effect of interaction between probiotics and prebiotics, and $e_{ij}$ is random error associated with each observation.

### RESULTS

#### Growth Performance

Significant differences in the growth performance were observed during the starter phase because of the main effects of probiotics ($P < 0.0001$) or prebiotics ($P = 0.005$) (Table 2). As shown in Table 2, during both starter and finisher phases, weight gain was significantly increased by probiotic supplementation ($P = 0.028$ or 0.04, respectively). In addition, it was also improved ($P = 0.01$) by dietary supplementation of MOS during the overall period; whereas, during the starter and finisher periods, it was unaffected by MOS addition. During the overall period, weight gain and FCR was influenced ($P < 0.05$) by prebiotic and it was better for MOS1 (Table 2). No interaction ($P > 0.05$) was observed for feed intake, weight gain, and FCR during the starter, finisher, and overall periods (Table 3). Feed intake was improved because of the main effect of probiotics ($P = 0.0001$) or MOS ($P = 0.005$) (Table 2).

#### Carcass Characteristics

There were no significant ($P > 0.05$) effects of probiotics ($P < 0.0001$) or prebiotics or their interactions on carcass, breast, thigh, heart, liver, and gizzard weight.
Antibody Titer Against Newcastle and Infectious Bursal Disease

Antibody titer for IBD was improved \((P = 0.026)\) by the interaction effect between probiotics and prebiotics, when compared with the control group. Antibody titer against ND was not affected by probiotic or prebiotic or their interactions \((P > 0.05)\) (Tables 6 and 7).

DISCUSSION

Results of feed intake in the starter phase were in line with Abdel-Raheem and Abd-Allah (2011) who observed that feed intake was improved by the supplementation of probiotics and prebiotics. The supplementation of probiotics decreased gastric emptying time, which leads to higher feed intake (Rahman et al., 2009; Abdel-Raheem and Abd-Allah, 2011). Similarly, some scientists observed that feed intake was not affected by the supplementation of probiotics (Sohail et al., 2012). By the supplementation of probiotics, GIT microbiota was balanced which is essential for the early development of the intestine which leads to higher feed intake in broilers during the starter phase (Hamasalim, 2016).

In contrast to these findings, some researchers observed that feed intake was decreased by the supplementation of prebiotics (Falaki et al., 2011) and probiotics (Amerah et al., 2013). Chen et al. (2015) found reduced feed intake by the addition of symbiotic (probiotic + prebiotic) in broiler diet. Mokhtari et al. (2010) found that feed intake was decreased by the addition of probiotics in the diet. According to Ferreira and Kussakawa (1999), the difference in results might be related to several factors such as birds and probiotic strain, sex, and dose rate. In broiler chickens, the use of probiotics (20 g/kg of ration) showed a positive impact on the growth rate. This is attributed to good intestinal health, leading to better nutrient digestion and absorption due to enhanced nutrients availability (Bedford, 2000).

Findings of weight gain were in line with results of Sohail et al. (2012) who observed that weight gain was not affected by symbiotics. However, individual effects of probiotics and prebiotics were similar to the findings of other scientists who reported that weight gain was increased with higher levels of probiotics (Sohail et al., 2012). Improvement in weight gain might be associated with the capability of probiotics to secrete enzymes such as amylase, protease, and lipase, which might improve the digestion rate of feed nutrients, which help in digestibility of starch, fat, and protein. So, increased availability of nutrients may be resulted in improved live weight gain of broiler (Bedford, 2000).

In contrast to these findings, Hanamanta et al. (2011) observed that weight gain was decreased by the addition of symbiotics. In addition, the weight gain was decreased during the first 21 d of the experiment by the addition of probiotics (Awad et al., 2015). Weight gain was not affected by supplementation of probiotics in broiler diet (Yousefi and Karkoodi, 2007). Similar to the findings of this trial, Sarangi et al. (2016) studied that FCR was not affected by the supplementation of symbiotics. Likewise, Awad et al. (2015) demonstrated that the use of probiotics in broiler diet did not affect FCR. Results of overall FCR were in line with Nikpiran et al. (2013) who observed better FCR with the addition of prebiotic in broiler diets. This can be explained by the reason that a more balanced biota population in the

| Items    | MOS0 | MOS1 | MOS1.5 | SEM | Sig  |
|----------|------|------|--------|-----|------|
| ND titer | 4.91 | 4.73 | 7.49   | 0.44| 0.347|
| IBD titer| 2.197| 3.388| 4.205  | 0.130| 0.176|

**Note:** Within a row, means sharing different superscripts differ significantly \((P < 0.05)\). Probiotic 0, 1, and 2 indicate inclusion of Protexin at the rate of 0, 1, and 2 g/kg of feed. MOS 0, 1, and 1.5 indicate inclusion of Active-MOS (mannan oligosaccharide) at the rate of 0, 1, and 1.5 g/kg of feed.

Abbreviations: Sig, significant; ND, Newcastle disease; IBD, infectious bursal disease.

**Table 6.** The main effect of probiotics or prebiotics on antibody body titer against Newcastle and infectious bursal disease of broiler.

| Items    | Pro0 | Pro1 | Pro2 |
|----------|------|------|------|
| MOS0     |      |      |      |
| MOS1     |      |      |      |
| MOS1.5   |      |      |      |

**Table 7.** The interaction effect of probiotics and prebiotics on antibody body titer against Newcastle and infectious bursal disease of broiler.

**Note:** Within a row, means sharing different superscripts differ significantly \((P < 0.05)\). Probiotic 0, 1, and 2 indicate inclusion of Protexin at the rate of 0, 1, and 2 g/kg of feed. MOS 0, 1, and 1.5 indicate inclusion of Active-MOS (mannan oligosaccharide) at the rate of 0, 1, and 1.5 g/kg of feed.

Abbreviations: Sig, significant; ND, Newcastle disease; IBD, infectious bursal disease.
gut due to availability of substrate could lead to a
greater efficiency in digestibility and utilization of feed,
resulting in an enhanced growth and improved FCR
(Bedford, 2000). Salianeh et al. (2011) also reported
that the FCR was decreased by the addition of prebiotics
in broiler diet when compared with the control group,
whereas addition of prebiotics did not have the same
effect as probiotics.

Similarly, Nikpiran et al. (2013) and Li et al. (2014)
found improved FCR by probiotics and prebiotics. Fallah et al. (2014) concluded that FCR was improved
by symbiotics in broiler and ostrich chicks. Improved
FCR might be due to maintaining normal microbiota
and better ileal digestibility by the addition of probiotic
and prebiotics (Rahman et al., 2009). In contrast to
these findings, mortality percentage was decreased by
symbiotic supplementation (Pelicano et al., 2005). Re-
sults of carcass, thigh, breast, organs (liver, heart, and
gizzard), and abdominal fat weights were in line with
Saied et al. (2015) who reported no effect of symbiotics
on carcass, breast, and thigh weight (Toghyani et al.,
2011), abdominal fat, and organs weight (Saied et al.,
2015). Similarly, use of probiotics did not affect thigh,
liver, heart, carcass, abdominal fat, and gizzard weights
(Shabani et al., 2012). Similar results were observed by
other scientists when prebiotics were added in broiler di-
ets (Yalcinkaya et al., 2008). Results of dressing percent-
age were in line with other researchers who reported that
the dressing percentage was increased by the addition of
symbiotics (Abdel-Raheem and Abd-Allah, 2011; Saied
et al., 2015). Carcass characteristics were improved by
the addition of prebiotic in broiler diet which might be
related to inhibition of colonization of intestinal patho-
gens and improved utilization of nutrients (protein and
ergy) in diet (Toghyani et al., 2011). The liver and
heart weights were decreased by the supplementation of
probiotics and prebiotics in the Japanese quail diet
(Nikpiran et al., 2013). Breast, gizzard, and thigh yield
were increased by symbiotics and MOS (Santin et al.,
2001). Liver and abdominal fat weights were reduced by
symbiotics (Abdel-Raheem and Abd-Allah, 2011; Saied
et al., 2015). On the other hand, dressing percentage
was not affected by symbiotics in quail’s diet. It has
been observed that the administration of probiotics mi-
croorganisms enhanced the protein availability. Symbi-
otic improved the nutrient uptake and also improved
the nitrogen stability, which can significantly affect the
carcass quality (Falaki et al., 2011).

Probiotics and prebiotics were supplemented with
poultry diet to prevent diseases (Elgeddawy et al.,
2020). The use of probiotics and prebiotics in poultry
feed can improve the immune status. Results of IBD titer
were according to Panda et al. (2000) who found higher
antibody titer against IBD by the addition of symbiotics
in broiler diets. However, Silva et al. (2009) studied that
titer against ND was not affected by symbiotics. Higher
antibody titer against IBD might be the result of regula-
tion of immunity by cytokines, which were secreted by
immune cells by the stimulation of probiotics microbes
(Lammers et al., 2003). Shahir et al. (2014) who found
that probiotic (5 × 10^10 colony forming units (cfu)
gram S. cerevisiae) improve the antibody responses to
ND of broiler chickens. In addition, An et al. (2008) illus-
trated that the antibody titers against ND in the broiler
chicks fed diets containing β-glucan (0.025, 0.05, or
0.1%) and Bacillus amyloliquefaciens (0.05, 0.1, or
0.2%) were significantly higher than in the control.
Houshmand et al. (2012) who found that antibody titer
to ND virus was higher in all probiotic- (Bacillus subtilis
and Clostridium butyricum) and prebiotic-treated (2 g/
kg) groups than in the control group, which indicated
a positive effect of the treated group on immunity.
Naseem et al. (2012) found that broiler chicks fed diets
supplemented with (50 or 150 g/ton) probiotic (Lactobac-
cillus species, Bifidobacterium, Streptococcus salivarius,
and E. faecium) had significantly higher antibody titers
against IBD. However, Findings of IBD titer were
against the results of Awad et al. (2015) who found
that antibody titer was decreased by the supplementation
of synbiotics. Silva et al. (2009) stated that the sup-
plementation of synbiotics did not affect the antibody
titer against IBD. This might be attributed to supple-
mentation of probiotics without prebiotics which act as
substrate for probiotic colonization.

**CONCLUSION**

On the basis of these results, it may be concluded that
the use of prebiotics and probiotics in broiler diets can
improve the growth rate. It may also be helpful in
improving the antibody titer against IBD in broilers
fed antibiotic free diets.

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**DISCLOSURES**

All authors declare that they do not have any conflicts
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