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## The effects of early feeding with different pre-starter diets on growth performance, morphological development and microorganism content of the small intestine of broiler chicks

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**ABSTRACT:** This study was conducted to compare the effects of feeding two different pre-starter diets to broiler chicks at two different feeding times on growth performance, slaughtering parameters, morphological development of the small intestine segments and the microorganism content of the ileum. A total of 600 day-old Ross 308 male broiler chicks were randomly distributed into five treatment groups consisting five replicates of 24 chicks each. The experimental treatments included SD10: broiler chicks were fed a corn-soybean meal-based starter diet for 0-10 days; 1PSD5: chicks were fed a first pre-starter diet based on corn-soybean meal supplemented with enzyme complex and symbiotic for 0-5 days, followed by a starter diet based on corn-soybean meal for 5-10 days; 1PSD10: chicks were fed the first pre-starter diet for 0-10 days; 2PSD5: chicks were fed a second pre-starter diet based on rice-soybean meal supplemented with enzyme complex and symbiotic for 0-5 days, followed by a starter diet based on corn-soybean meal for 5-10 days; and 2PSD10: chicks were fed the second pre-starter diet for 0-10 days. Broilers were fed a basal diet based on corn-soybean meal until the end of the experimental period. The 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly increased final body weight (BW) and BW gain, decreased feed intake (FI) and improved the feed conversion ratio (FCR) of broilers from day 0 to day 42 compared with the SD10 treatment. The lowest FI and the best FCR of broilers during the period of day 0 to day 42 were obtained with the 2PSD5 treatment. 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly enhanced the hot- and cold-carcass yields and villus heights (VHs), and decreased the crypt depths of small intestine segments of broilers on day 42. However, the experimental treatments did not significantly affect the *E.coli* and *Lactobacillus* contents of the ileum of broilers on day 42. In conclusion, feeding chicks with the pre-starter diet based on rice-soybean meal supplemented with enzyme complex and symbiotic for 0-5 days was the most effective treatment in terms of performance especially FI and FCR, carcass yields and VHs of small intestine segments of broilers.

**Keywords:** Newly hatched chick; pre-starter diet; feed additives; performance; small intestine

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## INTRODUCTION

The world population is predicted to reach over 9.8 billion people by the year 2050. In this context, the expected growth of consumption of broiler meat from 2010 to 2050 is estimated to be 173% (Borojerdi and Rajabzadeh, 2021). The fast-growing demand for broiler meat consumption as an animal protein source has made inevitable to increase broiler meat production (Evren, 2020). In the recent years, modern broiler chicks show a 50-fold increase in body weight (BW) by 40 days of age or an even shorter period of time (Tabeidian et al., 2016; Shafiei et al., 2018). As the growing period of modern broilers continues to shorten and the fast growth, the early nutritional practices of the chick become increasingly important to success (Tabeidian et al., 2016; Sasyte et al., 2018; Ravindran and Abdollahi, 2021). Recently, the early feeding studies had revealed that there is a strong positive correlation between the BW of the first week of rearing and the slaughter BW in broiler chickens (Ivanovich et al., 2017; Abou-Elnaga and Selim, 2018). Today, the first week after hatch represents 20-25% of the total production period considering the slaughter age of 42 days (Prabakar et al., 2016; Ravindran and Abdollahi, 2021). Although the first week post-hatch currently is a crucial part of the production period of broilers, chick's digestive capabilities are increasingly limited at this period (Sasyte et al., 2018; Shirani et al., 2018). At hatching, the digestive system of broiler chick is anatomically immature and its functional capacity is not fully development (Tabeidian et al., 2016). The digestive tract undergoes morphological (villus height (VH), villus surface area (VSA) and crypt depth (CD) etc. of the small intestine) and physiological (enzymes secretions of pancreas and small intestine) developments in the first week after hatch to allow proper digestion, absorption and utilization of ingested nutrients (Tabeidian et al., 2015).

Giving a pre-starter diet during the first week posthatch stimulates the morphological and physiological early development of the digestive system especially the small intestine (Tabeidian et al., 2016; Jha et al., 2019). The early development of the small intestine increases the availability of nutrients and thereby improved the subsequent performance and the health status of broilers (Tabeidian et al., 2015; Jha et al., 2019). Therefore, the choice of energy source feed ingredients and the use of feed additives in the pre-starter of broiler chicks can be of particular importance (Barekatain and Swick, 2016). In this respect, the type of starch and its digestion rate in different cereal grains

(wheat, corn, sorghum, rice) source in the pre-starter diets are important factors affecting broilers' performance. A positive correlation between starch digestion and broiler chickens' performance was pointed out (Nabizadeh et al., 2018). Among cereals, rice with its more digestibility for broiler chicks has been reported as a better cereal grain for the pre-starter diets than corn (Barekatain and Swick, 2016). Starch constitutes up to 70% of the rice grain (Barekatain and Swick, 2016) and rice starch has smaller granule size than corn starch (Tester et al., 2004). Small starch granules are generally digested more rapidly than larger granules (Franco et al., 1992). Rice starch is less protected from enzymatic attacks than corn starch (Tester et al., 2004). Consequently, endogenous enzymes will be more active and nutrient digestibility will be higher for rice than corn (Jiménez-Moreno et al., 2009). In addition, rice has less amylose and lower non-starch polysaccharide contents, which their high levels negatively influenced the digestibility of starch (Barekatain and Swick, 2016; Nabizadeh et al., 2018). Previous researches reported that diets based on rice than diets based on corn significantly increased the total tract apparent retention of nutrients (dry matter, organic matter, ether extract and AMEn) (Jiménez-Moreno et al., 2009) and improved feed conversion ratio from 1 to 21 d of age in broilers (González-Alvarado et al., 2007). The results of the study reported by Ebling et al. (2015) also suggested that better growth performance and nutrient digestibility can be obtained in broiler chicks and chickens if corn is replaced by rice in pre-starter diets.

Moreover, in recent decades, the inclusion of exogenous enzymes (amylase, lipase, protease), which cannot be produced at sufficient quantities and their activities in the digestive system of chicks are low, to the pre-starter diets also has come to the fore to improve nutrient digestion of young broiler chicks post-hatch (Ravindran and Abdollahi, 2021). Although the digestive enzymes secreted from the pancreas and small intestine of broiler chicks are present before hatching, their levels and activities are increased by feed intake only after hatching (Bar-Shira and Friedman, 2005). The activity of all enzymes increased with age, reaching maxima for amylase on day 5 and for trypsin and chymotrypsin around day 11 (Ravindran and Abdollahi, 2021). In addition, it is also reported that the supplementation of exogenous enzymes such as phytase, non-starch carbohydrates etc. is necessary to ameliorate the adverse effects of antinutrients, when feed ingredients containing an-

tinutrients were used in the pre-starter diets of broiler chicks post-hatch (Ravindran and Abdollahi, 2021). Thus, both the requirements for endogenous enzyme production to improve nutrients availability are reduced and the adverse effects of antinutrients in feed ingredients on the health and performance of young broiler chicks post-hatch are eliminated (Barekatain and Swick, 2016).

The gastrointestinal tract, especially the small intestine of the newly hatched broiler chicks is near-sterile and quite suitable for the establishment of pathogenic microorganisms in the small intestine (Corduk et al., 2013). The establishment of the bacterial populations occurs post-hatch. The number and diversity of these bacteria are changed by feeding and age and remained stable thereafter (Jha et al., 2019). Thus, early beneficial microbiota colonization of the small intestine in the post-hatch period is very critical for the future of well-being and growth performance of the broiler chicks during the rearing period (Corduk et al., 2013; Jha et al., 2019). Due to the above mentioned reasons, it is recommended to add synbiotics, the combination of probiotics and prebiotics, to the pre-starter diets in order to protect the microbial health of the small intestine (Jha et al., 2019).

In addition, the optimum feeding time of the pre-starter diet for broiler chicks also affects the growth performance and the development of the digestive system (Mahdavi et al., 2017). In this context, Mahdavi et al. (2017) reported that the optimum feeding time of pre-starter diet for broiler chicks grown to a target body weight appears to be 10 days. However, Durmuş (2018) pointed out that the pre-starter feeding during the first 5 days post-hatch did not influence the growth performance of broiler chickens at the end of the experiment.

To our best knowledge, there are previous studies on the use of either cereal grains with different starch digestibility or the supplementation of enzymes or synbiotics alone in the pre-starter diets of broiler chicks. Unlike the above studies, in the present research, we aimed to determine the appropriate pre-starter diet from the corn-soybean meal and rice-soybean meal based pre-starter diets with the exogenous enzymes and synbiotic combined supplementation and the optimum feeding time of this diet in terms of growth performance, slaughtering parameters and the small intestine parameters of broilers. Therefore, the objective of the present study was to compare the effects of feeding two different pre-starter diets to broiler chicks

at two different feeding times on growth performance, slaughtering parameters, morphological development of the small intestine segments and bacteria contents of the ileum of broilers.

## MATERIALS AND METHODS

### Animal care

The complete protocol was reviewed and approved by the Animal Care and Use Committee of Tokat Gaziosmanpasa University (Process no. 2020-HADYEK-22).

### Animals, diets, and experimental diet

On the day of hatching, a total of 600 day-old Ross 308 male broiler chicks were acquired from a commercial hatchery (Anadolu Ross, Ankara, Turkey). The broiler chicks were weighed, wing-banded and randomly distributed into 5 treatment groups with 5 replicates of 24 chicks each. From hatching until 6 week of age, the chicks were kept on floor pens bedded with fresh wood shavings as litter. Temperature was kept at 32°C for the first week, 28°C for the second week and gradually reduced, and after 27 days of age, temperature was remained at 21°C. A fluorescent lighting schedule of 23 h light and 1 h dark was used during the experiment with an average light intensity of 20 lux. The diets in mash form and drinking water were provided *ad libitum*.

All diets were formulated according to phase feeding practices as the broiler chickens advanced in age and weight, as recommended by the breeder (Ross 308, 2007); the starter phase lasted from day 0 to 10, the grower phase was from day 11 to 28 and the finisher phase was from day 29 to 42. The experimental treatments included: SD10: broiler chicks were fed a corn-soybean meal-based starter diet for 0-10 days; 1PSD5: chicks were fed a first pre-starter diet based on corn-soybean meal supplemented with enzyme complex and synbiotic for 0-5 days, afterwards they were fed a starter diet based on corn-soybean meal for 5-10 days; 1PSD10: chicks were fed the first pre-starter diet for 0-10 days; 2PSD5: chicks were fed a second pre-starter diet based on rice-soybean meal supplemented with enzyme complex and synbiotic for 0-5 days, afterwards they were fed a starter diet based on corn-soybean meal for 5-10 days; 2PSD10: chicks were fed the second pre-starter diet for 0-10 days. The ingredients and the nutrient composition of the first and second pre-starter diets are presented in Table 1. The phytase enzyme (Axtra®PHY 20000

TPT2) and Avizyme®1505X (amylase, xylanase and protease enzyme complex) were provided by Nutri-line Feed and Nutrient Additives (Istanbul, Turkey). Protease enzyme (Cibenza®EP150) was provided by Novus Feed Additives (Istanbul, Turkey). Anticoccidial (Maxiban) was provided by Elanco Animal Health (Istanbul, Turkey). Toxin binder (Mycofix) and a symbiotic (PoultryStar®) were also provided by Biomin Food, Agriculture and Livestock Limited Company (Istanbul, Turkey). The symbiotic (PoultryStar®) per kg contains *bifidobacterium animalis* ssp. *animals*, *lactobacillus salivarius* ssp. *salivarius* and *enterococcus faecium* in a ratio of 3:1:6, as well as fructooligosaccharide (Chicory roots). Broilers were fed the basal diets based on corn and soybean meal until the

end of the experimental period. The ingredients and the nutrient composition of the basal diets during the starter, grower and finisher phases also are presented in Table 2.

### Growth performance

During the 42-day experimental period, the growth performance of broilers was evaluated by recording their body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR). The body weight (BW) of broilers was recorded at the beginning of the experiment and on a weekly basis thereafter. FCR was calculated weekly as the amount of feed consumed per unit of BWG. Throughout the experiment, broilers were handled according to the principles for the

**Table 1.** The ingredients and the nutrient composition of the first and second pre-starter diets, %

Ingredients	The first pre-starter diet	The second pre-starter diet
Vegetable Oil	1.49	3.72
Corn	53.40	-
Rice	-	53.40
Corn Gluten Meal, % 62	0.40	4.80
Soybean Meal, % 48	30.20	25.795
Full-Fat Soybean	9.58	7.50
Enzyme Complex (amylase, xylanase and protease)	0.02	-
Protease Enzyme	-	0.025
Dicalcium Phosphate	2.35	2.52
DL-Methionine	0.37	0.30
Phytase Enzyme	0.01	0.01
Limestone	0.90	0.68
L-Lysine	0.24	0.29
L-Threonine	0.07	0.05
Anticoccidial	0.06	0.06
Choline Chloride	0.10	0.10
Symbiotic	0.10	0.10
Salt	0.36	0.30
Vitamin Premix <sup>1</sup>	0.25	0.25
Trace Mineral Premix <sup>2</sup>	0.10	0.10
Calculated Composition (%)		
Dry Matter	90.1	90.3
Crude Protein	23.0	23.0
ME, Kcal/kg	3025	3025
Ca	1.03	1.00
P available	0.50	0.50
Na	0.16	0.16
Methionine+Cystine	1.09	1.09
Lysine	1.44	1.44
Threonine	0.93	0.93
Tryptophan	0.31	0.30

<sup>1</sup>Vitamin premix/2.5 g/kg diet: 15 000 IU Vitamin A, 3 000 IU vitamin D3, 50 mg vitamin E, 5 mg vitamin K3, 3 mg vitamin B1, 6 mg vitamin B2, 5 mg vitamin B6, 0.03 mg vitamin B12, 25 mg niacin, 12 mg Ca-D-pantothenate, 1 mg folic acid, 0.05 mg D-biotin, 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride

<sup>2</sup> Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se

**Table 2.** The ingredients and the nutrient composition of the basal diets during the starter, grower and finisher phases, %

Ingredients	Starter Diet (0-10 days)	Grower Diet (11-28 days)	Finisher Diet (29-42 days)
Vegetable Oil	1.60	2.10	1.80
Corn	53.40	55.00	64.28
Soybean Meal, % 48	31.06	29.70	25.70
Full-Fat Soybean	9.26	10.00	5.00
Dicalcium Phosphate	2.32	0.70	0.79
Sodium Bicarbonate	-	-	0.12
DL-Methionine	0.37	0.33	0.26
Limestone	0.94	1.08	1.10
L-Lysine	0.22	0.34	0.21
L-Threonine	0.07	-	-
Anticoccidial	0.06	0.06	0.06
Choline Chloride	-	0.04	0.06
Salt	0.35	0.30	0.27
Vitamin Premix <sup>1</sup>	0.25	0.25	0.25
Trace Mineral Premix <sup>2</sup>	0.10	0.10	0.10
Calculated Composition (%)			
Dry Matter	90.1	90.1	90.2
Crude Protein	23	22	19
ME, Kcal/kg	3025	3150	3200
Ca	1.00	0.90	0.90
P available	0.50	0.45	0.45
Na	0.16	0.16	0.16
Methionine+Cystine	1.09	0.94	0.80
Lysine	1.44	1.29	1.00
Threonine	0.93	0.83	0.72
Tryptophan	0.31	0.29	0.25

<sup>1</sup>Vitamin Premix/2.5 g/kg diet: 15 000 IU Vitamin A, 3 000 IU vitamin D3, 50 mg vitamin E, 5 mg vitamin K3, 3 mg vitamin B1, 6 mg vitamin B2, 5 mg vitamin B6, 0.03 mg vitamin B12, 25 mg niacin, 12 mg Ca-D-pantothenate, 1 mg folic acid, 0.05 mg D-biotin, 2.5 mg apo-carotenoic acid ester; 400 mg choline chloride

<sup>2</sup> Trace Mineral Premix/kg diet: 80 mg Mn; 60 mg Fe; 60 mg Zn; 5 mg Cu; 0.2 mg Co; 1 mg I; 0.15 mg Se

care of animals in experimentation (Ross 308, 2007). Mortality was recorded daily.

### Slaughtering parameters

On each of the 5<sup>th</sup>, 10<sup>th</sup> and 42<sup>nd</sup> days of the experiment, the diets were withdrawn 6 hours ago prior to slaughter (Xue et al., 2021). After 6 hours feed withdrawal, two broilers from each replicates, whose BWs were similar to the group average, were selected from each of the treatment group (10 broilers per treatment) and a total of 50 broilers were slaughtered by severing the jugular vein on each of the aforementioned days to determine slaughtering parameters. A sodium pentobarbital injection (100 mg/kg) was applied as anesthesia to the experimental broilers before slaughter. The carcasses were immediately plucked, processed (removal of the head and feet), eviscerated (removal of the gastrointestinal tract), weighed and

then chilled overnight in a refrigerator (+4°C). The measurements included pre-slaughter BW, hot- and cold- carcass weights and weights of some internal organs. The weights of some internal organs (heart, liver, gizzard, proventriculus, spleen, pancreas and small intestine) were measured individually to the nearest  $\pm 0.001$  g. The weights of these internal organs and hot- and cold-carcass yields were calculated as a percentage of the pre-slaughter BW of broilers.

### Histomorphological measurements of small intestine segments

On each of the 5<sup>th</sup>, 10<sup>th</sup> and 42<sup>nd</sup> days of the experiment, the histomorphological parameters of the small intestine segments of 10 broilers slaughtered from each of the treatment groups were measured. Segments were removed from the duodenum, jejunum and ileum as follows: i) intestine from the giz-

zard to pancreatic and bile ducts was referred to as the duodenum; ii) midway was between the point of entry of the bile ducts and Meckel's diverticulum (jejunum); iii) 10 cm proximal to the ileo-caecal junction (ileum). Then, the samples were handled as described by Uni et al. (1998) to the morphological analysis of small intestine segments. First, the tissue samples were flushed with 0.9% saline solution to remove adherent intestinal contents and then fixed in 10% neutral buffered formaldehyde solution for 24 h (Çalik et al., 2017). The fixed duodenum, jejunum and ileum tissues were dehydrated in graded ethanol solutions, cleared in xylol and embedded in paraffin. The paraffin blocks were then sectioned at a thickness of 5  $\mu$ m with microtome (Rotary Leica RM2125RT). Cross sections were prepared and stained with hematoxylin and eosin staining in order to determine the histomorphometry of the small intestine segments. Sections were examined by light microscopy (Olympus binocular microscope BX53) and the villus height and the crypt depth of the small intestine segments were measured using a computer assisted image analysis described by Uni et al. (1998). Villus height (VH) was measured from the tip of villus to the crypt-villus junction, whereas the crypt depth (CD) was defined as the depth of the invagination between adjacent villi.

#### Determination of ileal bacterial populations

On each of the 10<sup>th</sup> and 42<sup>nd</sup> days of the experiment, the ileum of 10 broilers slaughtered from each of the treatment groups (from Meckel's diverticulum to the ileocecal junction) was removed individually. The ileum digesta were aseptically and individually collected in sterile 2-ml-tubes for ileal bacterial enumeration. One gram of ileum content was diluted 1:9 (wt/vol) with 0.85% sterile saline. The samples were diluted serially from 10<sup>-1</sup> to 10<sup>-3</sup> to determine *E. coli* concentration in the ileum and incubated on Eosin Methylene Blue (EMB) agar after incubation at 37°C for 48 h. The samples were diluted serially from 10<sup>-3</sup> to 10<sup>-4</sup> to determine *Lactobacillus* concentration in the ileum and anaerobically incubated on DeMan, Rogosa and Sharpe (MRS) agar after incubation at 37°C for 72 h. After incubation, microbial colonies were immediately counted and expressed as log<sub>10</sub> CFU/g.

#### Statistical analysis

The data obtained from the experiment were subjected to analysis of variance according to the randomized plots experimental design using SPSS (17.0)®

statistic package (SPSSWIN, 2007). Significant differences between treatment means were separated using Duncan's multiple range test (Duncan, 1955). All statements of significance were based on *P* value <0.05.

## RESULTS AND DISCUSSION

#### Growth performance

The effects of the experimental treatments on the growth performance of broilers are shown in Table 3.

There was no significant difference among the experimental treatments in terms of the initial body weight (BW) of broiler chicks (*P*>0.05).

1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly increased the BW of broilers on the 5<sup>th</sup> (*P*<0.001), 10<sup>th</sup> (*P*<0.001) and 42<sup>nd</sup> days (*P*<0.05) of the experiment compared with the SD10 treatment. In particular, the highest final BW of broilers on the 42<sup>nd</sup> day was obtained with the 2PSD5 treatment.

Our results highlight that the 2PSD5 and 2PSD10 treatments significantly increased the final BW of broilers compared with the SD10 treatment. There is no data on the effect of substituting rice for corn in pre-starter diets on the final BW of broilers. However, this finding is partially in agreement with the result reported by Nanto et al. (2012) who showed that the significantly high final BW in broilers was obtained when corn was completely replaced by dehulled paddy rice in the diets throughout the experiment covering the period from day 11 to day 28. On the contrary, Edwin et al. (2002) found no significant differences in the final BW of broilers that were fed on a diet with a 75% replacement of corn with broken rice.

Although there is little to no literature on the addition of enzyme mixture to a corn-soybean meal-based pre-starter diet, our result related to the final BW of broilers is partially in agreement with the findings reported by Café et al. (2002). They stated that the addition of enzyme mixture (Avizyme) to a corn-soybean meal-based diet during all experiment period resulted in a significant improvement in the final BW of broilers. However, Kirkpinar et al. (2018) and dos Santos Neto et al. (2021) reported that the supplementation of enzyme mixture to the diet based on corn-soybean meal did not influence the final BW of broilers compared with the negative control diet.

The 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly increased the BWG of broilers from day 0 to 5 (*P*<0.001), from day 0 to 10

( $P<0.001$ ) and from day 0 to 42 ( $P<0.05$ ) of the experiment compared with the SD10 treatment. In particular, the highest BWG of broilers throughout the experiment was obtained with the 2PSD5 treatment (Table 3).

Our finding that the 2PSD5 and 2PSD10 treatments significantly increased the BWG of broilers from day 0 to 42 compared with the SD10 treatment concurs with the result reported by Ebling et al. (2015). They stated that broilers fed on the diet where corn was completely replaced with white rice in the pre-starter diet from day 1 to 7 had the higher BWG between 1 to 33 days compared with the control diet based on corn-soybean meal. Likewise, Nabizadeh et al. (2018) highlighted that the BWG of chickens during age 1 to 42 days was linearly and significantly higher with increased inclusion of broken rice (6%, 12% and 18%) in a pre-starter diet from age 1 to 10 days compared with the control diet without broken rice.

In this study, the 1PSD5 and 1PSD10 treatments during the pre-starter period significantly enhanced the BWG of broilers from day 0 to 5, day 0 to 10 and day 0 to 42 compared with the SD10 treatment. Our result concurs with the finding reported by Lima et al. (2011) who showed that the supplementation of an enzyme complex at the level of 300 g/tonne to the pre-starter diet based on corn-soybean meal increased the BWG of 7 day-old broilers. Although there is little research on the effect of the combined supplementation of enzyme mixture or enzyme mixture+synbiotic to pre-starter diets on the BWG of broilers, our finding is partially in agreement with the result of Kırkpınar et al. (2018). They stated that the combined supplementation of enzyme mixture+probiotic to a diet based on corn-soybean meal significantly increased the BWG of broilers from day 1 to 42 compared with the negative control diet. On the contrary, dos Santos Neto et al. (2021) reported that the supplementation of enzyme mixture to a diet based on corn-soybean

meal throughout the experiment did not influence the BWG of broilers during the day 1 to 42 compared with the control diet without enzyme mixture.

As shown in Table 3, the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly enhanced the FI of broilers from day 0 to 5 ( $P<0.001$ ) and from day 0 to 10 ( $P<0.01$ ) compared with the SD10 treatment. Moreover, the FI of broilers from day 0 to 42 was significantly decreased by the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments when compared with the SD10 treatment ( $P<0.05$ ). In addition, 2PSD5 significantly decreased the FI of broilers during the day 0 to 42 compared with the SD10, 1PSD5, 1PSD10 and 2PSD10 treatments ( $P<0.05$ ).

Our result related to the FI of broilers during day 0 to 42 concurs with the finding reported by Nabizadeh et al. (2018) who stated that the FI of broilers during age 1 to 42 days was significantly decreased with increasing inclusion of broken rice (6%, 12% and 18%) in a pre-starter diet from age 1 to 10 days compared with the control diet without broken rice. Contrary to our findings, Ebling et al. (2015) stated that the complete replacement of corn with broken rice in the pre-starter diet from age 1 to 7 days did not influence the FI of broilers during days 1 to 33 compared with the control diet based on corn-soybean meal.

In our study, the 1PSD5 and 1PSD10 treatments during the pre-starter period significantly decreased the FI of broilers from day 0 to 42 compared with the SD10 treatment. There is no research on the effect of the combined supplementation of enzyme mixture or enzyme mixture+synbiotic to the pre-starter diets on the FI of broilers. In contrast, our finding is not in agreement with the result of Kırkpınar et al. (2018) who showed that the combined supplementation of enzyme mixture+probiotic to a diet based on corn-soybean meal did not affect the FI of broilers from age 1 to 42 days compared with the control diet

**Table 3.** The effects of the experimental treatments on the growth performance of broilers

Experimental Treatments	Body Weight (BW), g				Body Weight Gain (BWG), g		Feed Intake (FI), g				Feed Conversion Ratio (FCR), g:g		
	Initial	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	0 to 5 d	0 to 10 d	0 to 42 d	0 to 5 d	0 to 10 d	0 to 42 d	0 to 5 d	0 to 10 d	0 to 42 d
SD10	41.81	82.86 <sup>c</sup>	174.76 <sup>c</sup>	2445.45 <sup>c</sup>	41.05 <sup>d</sup>	132.94 <sup>c</sup>	2403.64 <sup>c</sup>	53.98 <sup>c</sup>	196.03 <sup>c</sup>	4379.77 <sup>a</sup>	1.32 <sup>a</sup>	1.48 <sup>a</sup>	1.82 <sup>a</sup>
1PSD5	41.75	93.04 <sup>ab</sup>	188.78 <sup>b</sup>	2522.95 <sup>ab</sup>	51.30 <sup>b</sup>	147.04 <sup>b</sup>	2481.21 <sup>ab</sup>	60.72 <sup>b</sup>	210.32 <sup>ab</sup>	4282.63 <sup>b</sup>	1.19 <sup>b</sup>	1.43 <sup>b</sup>	1.73 <sup>b</sup>
1PSD10	41.75	90.67 <sup>ab</sup>	194.44 <sup>b</sup>	2536.34 <sup>ab</sup>	48.92 <sup>c</sup>	152.70 <sup>b</sup>	2494.59 <sup>ab</sup>	58.52 <sup>b</sup>	213.39 <sup>a</sup>	4302.45 <sup>b</sup>	1.20 <sup>b</sup>	1.40 <sup>b</sup>	1.73 <sup>b</sup>
2PSD5	41.73	96.86 <sup>a</sup>	190.43 <sup>b</sup>	2587.08 <sup>a</sup>	55.13 <sup>a</sup>	148.70 <sup>b</sup>	2545.35 <sup>a</sup>	64.38 <sup>a</sup>	208.98 <sup>ab</sup>	4183.94 <sup>c</sup>	1.17 <sup>b</sup>	1.41 <sup>b</sup>	1.64 <sup>d</sup>
2PSD10	41.81	94.19 <sup>ab</sup>	214.15 <sup>a</sup>	2548.60 <sup>ab</sup>	52.38 <sup>b</sup>	172.34 <sup>a</sup>	2506.79 <sup>ab</sup>	64.70 <sup>a</sup>	222.23 <sup>a</sup>	4256.58 <sup>b</sup>	1.24 <sup>b</sup>	1.29 <sup>c</sup>	1.70 <sup>bc</sup>
SEM	0.211	1.167	3.230	13.387	1.135	3.194	18.487	0.983	2.391	26.919	0.015	0.019	0.016
P-value	1.000	0.000	0.000	0.016	0.000	0.000	0.017	0.000	0.002	0.024	0.002	0.026	0.001

a-d Values in the same column not sharing a common superscript differ significantly (\* $P<0.05$ ; \*\* $P<0.01$ ; \*\*\* $P<0.001$ )

SEM: Standard Error of the Mean

without any feed additives.

The FCR of broilers from day 0 to 5 ( $P<0.01$ ), day 0 to 10 ( $P<0.05$ ) and day 0 to 42 ( $P<0.01$ ) was significantly decreased by the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments compared with the SD10 treatment. In addition, the lowest FCR of broilers from day 0 to 42 was obtained by the 2PSD5 treatment, followed by the 2PSD10 treatment (Table 3).

Our finding that the 2PSD5 and 2PSD10 treatments significantly decreased the FCR of broilers from day 0 to 42 compared with the SD10 treatment concurs with the result reported by Ebling et al. (2015). They found that broilers fed on the diet where corn was totally replaced with white rice in the pre-starter diet from age 1 to 7 days had significantly lower FCR at days 1 to 33 compared with the control diet based on corn-soybean meal. Likewise, Nabizadeh et al. (2018) reported that the FCR of chickens during age 1 to 42 days was linearly and significantly decreased with increasing inclusion of broken rice (6%, 12% and 18%) in a pre-starter diet from age 1 to 10 days compared with the control diet without the broken rice.

In this study, the 1PSD5 and 1PSD10 treatments during the pre-starter period significantly decreased the FCR of broilers from day 0 to 5, day 0 to 10 and day 0 to 42 compared with the SD10 treatment. There is little research on the effect of the combined supplementation of enzyme mixture to the pre-starter diets on the FCR of broilers. Furthermore, our finding related to the FCR of 5 day-old broiler chicks was not consistent with finding by Lima et al. (2011) who stated that the supplementation of enzyme complex up to 300 g/tonne level did not affect the FCR of 7-day-old broilers. Our finding partially concurs with the result reported by Zanella et al. (1999) who highlighted that the supplementation of 0.1% Avizyme to broiler diet based on corn-soybean meal throughout the experiment significantly improved the FCR of broilers from day 0 to 45 compared with a diet without the enzyme complex supplement. Contrary to our result, Café et al. (2002) and dos Santos Neto et al. (2021) showed that the addition of 0.1% Avizyme to broiler diet based on corn-soybean meal had no significant effect on FCR of broiler chicks during all experimental period.

In summary, nutrients' utilization might be increased by the exogenous supplementation of enzymes, which have limited enzyme capacity (amylase and protease) and degradation of specific bonds in ingredients not hydrolyzed due to lack of endogenous

digestive enzymes (xylanase and phytase) in the digestive system of young chicks, to the pre-starter diets based on corn-soybean meal and rice-soybean meal for young broiler chicks for 0-5 days and 0-10 days in this study (Ravindran and Abdollahi, 2021). As a result of these, the availability of energy, starch, protein and phosphorus was improved and the adverse effects of non-starch polysaccharides were reduced, thus the utilization of dietary nutrients was improved to contribute to better performance of broilers in the early stages of life and throughout rearing (Barekatain and Swick, 2016; dos Santo Neto et al., 2021; Alqhtani et al., 2022). The probiotic addition to the pre-starter diets based on corn-soybean meal and rice-soybean meal as well as the exogenous enzyme supplementation in our study stimulates the health of the small intestine through development of its beneficial bacteria contents, improvement of its histomorphological structure and by encouraging immune function of young broiler chicks (Ravindran and Abdollahi, 2022). The positive synergistic effects of the aforementioned feed additives supplemented to the pre-starter diets might have a greater effect on the growth performance of broilers both at the early stages of life and throughout rearing.

Moreover, in this study feeding chicks with the second pre-starter diet based on rice-soybean meal for 0-5 days in particular, numerically increased final BW and BWG, significantly decreased FI and improved FCR of broilers from day 0 to 42 compared with those of broilers fed the first pre-starter diet based on corn-soybean meal for 0-5 days and 0-10 days. This effect might be attributed to the better digestion of rice than corn in broiler chicks because rice has smaller granule size, high starch content, lower amylose, lower crude fiber and less non-starch polysaccharide content than those in corn (Filguera et al., 2014; Ashour et al., 2015; Ebling et al., 2015; Nabizadeh et al., 2018). Also, starch makes up to 70% of the rice grain and rice is more crystalline in natural form and less protected from enzymatic attack than corn starch due to its lower encapsulation of starch (Jiménez-Moreno et al., 2009; Nabizadeh et al., 2018). In conclusion, endogenous enzymes are more active and the digestion of nutrients in the gastrointestinal tract of broilers is higher for rice than corn (Jiménez-Moreno et al., 2009).

### Slaughtering parameters

The effects of the experimental treatments on the slaughtering parameters of broilers are shown in Ta-

ble 4.

The experimental treatments did not significantly affect the relative weights (RWs) of heart, liver, proventriculus, pancreas, spleen, bursa fabricius and small intestine of broilers on day 42 ( $P>0.05$ ).

The finding related to the RW of liver of broilers in this study is partially consistent with the study by González-Alvarado et al. (2007) where no influence of the total substitution of corn with broken rice in the diet from day 0 to 21 on the RW of liver of broilers on day 21 was observed.

Contrary to our finding related to the RW of proventriculus of broilers, González-Alvarado et al. (2007) stated that the total replacement of corn with rice in the diet from age 1 to 21 days significantly decreased the RW of proventriculus of broilers on day 21 compared with the control diet based on a corn-soybean meal.

Similarly, Kirkpinar et al. (2018) found no difference in the RW of small intestine of broilers fed corn-soybean meal-based diets supplemented with prebiotic+probiotic, prebiotic+enzyme mixture and probiotic+enzyme mixture at 42 days of age.

As shown in Table 4, the 2PSD5 and 2PSD10 treatments significantly decreased the RW of the gizzard of broilers when compared with the SD10, 1PSD5 and 1PSD10 treatments ( $P<0.001$ ). This finding is in agreement with the results of Brum Júnior et al. (2007), González-Alvarado et al. (2007) and Nanto et al. (2012) who indicated that the gizzard weight of broilers decreased when substituting corn with broken rice, raw rice and dehulled paddy rice. This effect might be related to lower fiber and non-starch polysaccharide and the higher starch contents of rice compared with corn (Filgueira et al., 2014). Our data concurs with that of Rama Rao et al. (2000) who reported a heavier gizzard in broiler breeder

chickens fed a corn-based diet compared with broilers fed a rice-based diet. The reduction of gizzard weight observed in chickens fed a rice-based diet might be attributed to less mechanical stimulation associated with less digesta contents in the gizzard due to its lower fiber content as compared with chickens fed a corn-based diet, because less fiber is retained in the stomach for a shorter time.

The hot-carcass and cold-carcass yields of broilers at age 42 days were significantly increased by the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments compared with those of broilers fed the SD10 treatment ( $P<0.05$ ). This finding is consistent with the result of Alqhtani et al. (2022) who reported that the supplementation of enzyme mixture into a corn-soybean meal-based diet significantly enhanced carcass yield of broilers at 35 days compared with that of broilers fed a corn-soybean meal diet without enzyme supplementation. Contrary to the result in this study, dos Santos Neto et al. (2021) found no difference between carcass yields of broilers fed a corn-soybean meal-based diet with enzyme mixture (xylanase, amylase, protease,  $\alpha$ -galactosidase and phytase) or without enzyme mixture supplementation.

In this study, the improvement in the carcass yields of broilers can be partially explained by the increased final BW of broilers fed the pre-starter diets based on corn-soybean meal and rice-soybean meal supplemented with the exogenous enzymes for 0-5 days and 0-10 days, since these exogenous enzymes contain several enzymatic activities (xylanase, amylase, protease and phytase). It was reported that these exogenous enzymes can target multiple nutrients within feeds, thus having a greater effect than individual enzymes, which target one substrate (Alqhtani et al., 2022).

The supplementation of exogenous enzymes such as amylase, xylanase, protease and phytase to the

**Table 4.** The effects of the experimental treatments on the slaughtering parameters of broilers, %1

Experimental Treatments	Heart	Liver	Proventriculus	Gizzard	Pancreas	Spleen	Bursa Fabricius	Small Intestine	Hot Carcass Yield	Cold Carcass Yield
SD10	0.44	1.66	0.39	2.47 <sup>a</sup>	0.23	0.12	0.23	2.98	72.00 <sup>c</sup>	68.40 <sup>c</sup>
1PSD5	0.47	1.70	0.39	2.04 <sup>ab</sup>	0.23	0.12	0.27	2.81	73.26 <sup>b</sup>	69.59 <sup>b</sup>
1PSD10	0.45	1.68	0.38	2.01 <sup>ab</sup>	0.23	0.13	0.27	2.88	73.64 <sup>b</sup>	69.96 <sup>b</sup>
2PSD5	0.45	1.73	0.38	1.89 <sup>c</sup>	0.23	0.11	0.22	2.76	74.55 <sup>a</sup>	70.83 <sup>a</sup>
2PSD10	0.45	1.70	0.34	1.91 <sup>c</sup>	0.22	0.12	0.23	2.73	74.11 <sup>a</sup>	70.40 <sup>a</sup>
SEM	0.007	0.020	0.007	0.047	0.006	0.004	0.011	0.045	0.266	0.252
P-value	0.873	0.870	0.085	0.000	0.976	0.702	0.505	0.393	0.020	0.020

<sup>a-c</sup> Values in the same column not sharing a common superscript differ significantly (\* $P<0.05$ ; \*\*\* $P<0.001$ )

SEM: Standard Error of the Mean, <sup>1</sup> Data are means of 10 individual chickens per treatment group

pre-starter diets based on corn-soybean meal and rice-soybean meal might improve nutrient digestion due to better access of the endogenous enzymes to the cellular content and hydrolysis of arabinoxylans and phytin-phosphorus of the cellular wall of the feed ingredients (Giacobbo et al., 2021). Thus, combined supplementation of exogenous enzymes with several enzyme activities into diets of young broiler chicks that still have an immature digestive tract and low enzyme production, might majorly improve nutrients' utilization (proteins, carbohydrates, starch and phosphorus) and growth performance not only in the pre-starter phase, but throughout the rearing period (dos Santos Neto et al., 2021). Moreover, this improvement in the carcass yields of broilers fed a diet based on rice-soybean meal supplemented with several exogenous enzymes was superior than those of broilers with a diet based on corn-soybean meal supplemented with several exogenous enzymes, because rice has lower fiber and non-starch polysaccharide levels and higher starch contents than those of corn (Filgueira et al., 2014).

### Histomorphological parameters of small intestine segments

#### Villus height

The effects of the experimental treatments on the villus heights (VHs) of small intestine segments of broilers are shown in Table 5.

1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly increased the VH of the duodenum, jejunum and ileum of broilers on the 5<sup>th</sup> ( $P<0.001$ ), 10<sup>th</sup> ( $P<0.001$ ) and 42<sup>nd</sup> ( $P<0.001$ ) days of the experiment compared with that of the SD10 treatment. Furthermore, the VH of duodenum and ileum of broilers on the 5<sup>th</sup>, 10<sup>th</sup> and 42<sup>nd</sup> days of the experiment was significantly increased by the 2PSD5 and 2PSD10

treatments compared with those of the 1PSD5, 1PSD10 and SD10 treatments ( $P<0.001$ ). This result concurs with the finding reported by Nabizadeh et al. (2018) who stated that the VH of duodenum and ileum of broilers on the 5<sup>th</sup> day was significantly increased with a greater inclusion of broken rice (12% and 18%) in pre-starter diet from age 1 to 10 days compared with the control diet based on corn-soybean meal. Moreover, in the same study, the high inclusion of broken rice (18%) in pre-starter diet from age 1 to 10 day significantly increased the VH of ileum of broilers on the 10<sup>th</sup> day of the experiment compared with the control diet based on corn-soybean meal. This increase might be attributed to the better digestion of rice starch than corn starch in the small intestine of broiler chicks because rice has smaller granule size, higher starch content, and lower amylose, crude fiber and non-starch polysaccharide contents than those in corn (Filgueira et al., 2014; Ashour et al., 2015; Ebling et al., 2015; Nabizadeh et al., 2018). Consequently, this might be due to the fact that a rice-based diet was used as an energy source in the development of small intestine villi, since rice-based diet resulted in a high metabolisable energy availability in broilers (Tang et al., 2014; Tabeidian et al., 2015).

The exogenous supplementation of enzymes such as amylase, xylanase, protease and phytase to the pre-starter diets based on corn-soybean meal and rice-soybean meal for young broiler chicks for 0-5 days and 0-10 days in this study might have increased the VH of small intestine segments of broilers by increasing nutrient digestion and decreasing viscosity of the small intestine content by diminishing the antinutritional effect of non-starch polysaccharides (Tang et al., 2014; Tabeidian et al., 2015; Giacobbo et al., 2021). Our finding is in agreement with the result of Çalik et al. (2017) who stated that symbiotic

**Table 5.** The effects of the experimental treatments on the villus heights of duodenum, jejunum and ileum of broilers slaughtered on each of the 5th, 10th and 42nd days of the experiment,  $\mu\text{m}$

Experimental Treatments	Villus Height (VH)								
	Duodenum				Jejunum				Ileum
5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	
SD10	734.50 <sup>d</sup>	941.50 <sup>d</sup>	964.65 <sup>d</sup>	497.50 <sup>c</sup>	955.50 <sup>c</sup>	982.15 <sup>c</sup>	476.50 <sup>e</sup>	567.00 <sup>c</sup>	893.00 <sup>e</sup>
1PSD5	853.00 <sup>c</sup>	968.00 <sup>c</sup>	1003.00 <sup>c</sup>	659.00 <sup>b</sup>	979.00 <sup>b</sup>	999.00 <sup>bc</sup>	581.91 <sup>c</sup>	640.00 <sup>b</sup>	958.00 <sup>d</sup>
1PSD10	866.19 <sup>b</sup>	989.05 <sup>b</sup>	1029.14 <sup>b</sup>	662.86 <sup>b</sup>	975.71 <sup>b</sup>	1012.00 <sup>b</sup>	500.50 <sup>d</sup>	645.00 <sup>b</sup>	984.67 <sup>c</sup>
2PSD5	881.00 <sup>a</sup>	1164.00 <sup>a</sup>	1198.29 <sup>a</sup>	692.86 <sup>a</sup>	980.00 <sup>b</sup>	1038.86 <sup>a</sup>	644.76 <sup>a</sup>	687.50 <sup>a</sup>	1025.85 <sup>a</sup>
2PSD10	876.19 <sup>a</sup>	1184.29 <sup>a</sup>	1182.00 <sup>a</sup>	691.50 <sup>a</sup>	1022.86 <sup>a</sup>	1019.71 <sup>b</sup>	600.00 <sup>b</sup>	690.48 <sup>a</sup>	1009.48 <sup>b</sup>
SEM	5.759	10.637	9.954	7.494	3.243	3.038	6.402	4.642	4.838
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

<sup>a-e</sup> Values in the same column not sharing a common superscript differ significantly (\*\* $P<0.001$ )

SEM: Standard Error of Mean, <sup>1</sup> Data are means of 10 individual chickens per treatment group

supplementation to a corn-soybean meal-based diet significantly enhanced the VH of ileum of broilers on the 42<sup>nd</sup> day compared with that of a diet without the synbiotic supplementation. This could be due to the beneficial effects of synbiotics on the intestinal microflora, which reduces pathogenic infections in the mucosa by maintaining the structural integrity of the small intestine and increasing the area of absorption of the small intestine (Bogucka et al., 2019).

### Crypt depth

The effects of the experimental treatments on the crypt depths (CDs) of small intestine segments of broilers are shown in Table 6.

The 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly decreased the CD of duodenum of broilers on the 5<sup>th</sup> ( $P<0.001$ ), 10<sup>th</sup> ( $P<0.001$ ) and 42<sup>nd</sup> ( $P<0.001$ ) days compared with the SD10 treatment. The CD of jejunum on the 5<sup>th</sup> day was significantly ( $P<0.001$ ) reduced with 2PSD5 and 2PSD10 treatments when compared with the other treatments, although the 1PSD10, 2PSD5 and 2PSD10 treatments significantly ( $P<0.001$ ) decreased the CD of jejunum of broilers on the 10<sup>th</sup> day of the experiment compared with those of the SD10 and 1PSD5 treatments (Table 6). In addition, the CD of jejunum on the 42<sup>nd</sup> day was significantly ( $P<0.001$ ) reduced by the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments compared with that of the SD10 treatment. Furthermore, 1PSD10, 2PSD5 and 2PSD10 treatments significantly ( $P<0.001$ ) reduced the CD of ileum of broilers on the 5<sup>th</sup> day compared with those of SD10 and 1PSD5 treatments, while the CD of ileum of broilers on the 10<sup>th</sup> and 42<sup>nd</sup> days was significantly ( $P<0.001$ ) reduced by 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments when compared with the SD treatment. Our findings related to the CD of duode-

num, jejunum and ileum of broilers on the 5<sup>th</sup> day are not in agreement with the result of Nabizadeh et al. (2018) who reported that the CD of duodenum, jejunum and ileum of broilers on the 5<sup>th</sup> day was not influenced by an increasing inclusion of broken rice (6%, 12% and 18%) in pre-starter diet from age 1 to 10 days compared with the control diet based on corn-soybean meal. However, our results related to the CD of duodenum, jejunum and ileum of broilers on the 10<sup>th</sup> day partially concur with the findings of Nabizadeh et al. (2018). They stated that an increasing inclusion of broken rice (12% and 18%) in a pre-starter diet from day 1 to 10 significantly reduced the CD of only duodenum of broilers on the 10<sup>th</sup> day, but did not affect the CD of jejunum and ileum of broilers on the 10<sup>th</sup> day, compared with the control diet based on corn-soybean meal.

Furthermore, our result is not in agreement with the finding of Shakouri et al. (2008) and Giacobbo et al. (2021) who reported that the supplementation of enzyme mixture to a corn-soybean meal-based diet did not affect the CD of jejunum of broilers at the end of the experiment compared with that of broilers fed the control diet without the enzyme mixture supplementation. Our findings are also not in agreement with the results of Salehimanesh et al. (2016) and Çalık et al. (2017) who stated that the synbiotic supplementation to the diet based on corn-soybean meal did not affect the CD of ileum of broilers on day 42 compared with broilers fed the diet without the synbiotic supplementation.

In summary, feeding chicks with a pre-starter diet based on corn-soybean meal and rice-soybean meal supplemented with enzyme complex (amylase, protease, xylanase and phytase) and synbiotic for 0-5 days and 0-10 days significantly reduced the CDs of duodenum, jejunum and ileum of broilers on day 42

**Table 6.** The effects of the experimental treatments on the crypt depth of duodenum, jejunum and ileum of broilers slaughtered on each of the 5th, 10th and 42nd days of the experiment,  $\mu\text{m}$

Experimental Diets	Crypt Depth (CD)								
	Duodenum				Jejunum		Ileum		
	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d	5 <sup>th</sup> d	10 <sup>th</sup> d	42 <sup>nd</sup> d
SD10	217.0 <sup>a</sup>	211.4 <sup>a</sup>	201.0 <sup>a</sup>	173.0 <sup>a</sup>	183.5 <sup>a</sup>	172.5 <sup>a</sup>	172.4 <sup>a</sup>	173.0 <sup>a</sup>	174.5 <sup>a</sup>
1PSD5	203.0 <sup>b</sup>	203.5 <sup>b</sup>	183.5 <sup>b</sup>	170.5 <sup>a</sup>	182.5 <sup>a</sup>	168.1 <sup>b</sup>	170.0 <sup>a</sup>	141.5 <sup>b</sup>	157.5 <sup>b</sup>
1PSD10	191.5 <sup>c</sup>	191.0 <sup>c</sup>	181.9 <sup>b</sup>	170.0 <sup>a</sup>	178.1 <sup>b</sup>	163.5 <sup>b</sup>	132.5 <sup>b</sup>	133.0 <sup>c</sup>	147.5 <sup>c</sup>
2PSD5	191.0 <sup>c</sup>	188.0 <sup>c</sup>	158.0 <sup>c</sup>	146.0 <sup>b</sup>	128.6 <sup>c</sup>	151.0 <sup>c</sup>	112.0 <sup>c</sup>	115.2 <sup>d</sup>	135.2 <sup>d</sup>
2PSD10	171.0 <sup>d</sup>	141.0 <sup>d</sup>	136.2 <sup>d</sup>	112.0 <sup>c</sup>	121.0 <sup>c,d</sup>	144.8 <sup>d</sup>	104.0 <sup>d</sup>	112.9 <sup>d</sup>	128.6 <sup>e</sup>
SEM	1.924	2.607	2.400	2.560	2.879	1.288	2.940	2.310	2.080
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

<sup>a-e</sup> Values in the same column not sharing a common superscript differ significantly (\*\* $P<0.001$ )

SEM: Standard Error of Mean, <sup>1</sup> Data are means of 10 individual chickens per treatment group

**Table 7.** The effects of the experimental treatments on the *E.coli* and *Lactobacillus* contents of ileum of broilers slaughtered on each of the 0<sup>th</sup> and 42<sup>nd</sup> days of the experiment, log10 CFU/g<sup>1</sup>

Experimental Treatments	<i>E. coli</i>		<i>Lactobacillus</i>	
	10 <sup>th</sup> day	42 <sup>nd</sup> day	10 <sup>th</sup> day	42 <sup>nd</sup> day
SD10	5.68	6.57	6.58 <sup>b</sup>	7.90
1PSD5	5.53	6.79	6.87 <sup>a</sup>	7.52
1PSD10	5.60	6.81	6.89 <sup>a</sup>	7.42
2PSD5	5.57	6.96	6.89 <sup>a</sup>	7.71
2PSD10	5.56	6.90	6.87 <sup>a</sup>	7.46
SEM	0.081	0.092	0.030	0.120
P-value	0.984	0.832	0.000	0.781

a-b Values in the same column not sharing a common superscript differ significantly (\*\*P<0.001)

SEM: Standard Error of Mean, <sup>1</sup> Data are means of 10 individual chickens per treatment group

compared with the starter diet based on corn-soybean meal without enzyme complex and probiotic. This reduction might be attributed to a decrease in viscosity and the presence of toxins in small intestine segments of broilers by diminishing their antinutritional effect due to hydrolysis of non-starch polysaccharides such as arabinoxylans of the cellular wall in the feed ingredients with dietary supplementation of enzyme complex and probiotic in combination. A decreased viscosity of the contents of the small intestine segments of broilers caused to a better development of the layer of the villus in the small intestine segments and a reduced crypt cell proliferation rate to result in an increased absorptive area in the digestive tract (Tabeidian et al., 2015; Giacobbo et al., 2021).

#### *E. coli* and *Lactobacillus* contents of the ileum

The effects of the experimental treatments on the *E.coli* and *Lactobacillus* contents of ileum of broilers slaughtered on the 10<sup>th</sup> and 42<sup>nd</sup> days are given in Table 7.

The experimental treatments did not influence the *E.coli* content of the ileum of broilers on the 10<sup>th</sup> ( $P>0.05$ ) and 42<sup>nd</sup> ( $P>0.05$ ) days and *Lactobacillus* content of their ileum on the 42<sup>nd</sup> day ( $P>0.05$ ). However, the 1PSD5, 1PSD10, 2PSD5 and 2PSD10 treatments significantly increased the *Lactobacillus* content of ileum of broiler on the 10<sup>th</sup> day compared with that of the SD10 treatment ( $P<0.001$ ). Our finding related to the *Lactobacillus* content of ileum of broiler on the 10<sup>th</sup> day is not in agreement with the result of Nabizadeh et al. (2018). They reported that the inclusion of broken rice in a pre-starter diet did not have a significant effect on the *Lactobacillus* content of ileum of broilers at age 10 days compared with the control diet based on corn-soybean meal. Our results related to the *E.coli* and *Lactobacillus* contents

of ileum of broilers on the 42<sup>nd</sup> day concur with the findings of Salehimanesh et al. (2016) who reported that probiotic supplementation to the diet based on corn-soybean meal did not affect the *E.coli* and *Lactobacillus* contents of ileum of broilers on day 42 when compared with a diet without the probiotic supplementation.

#### CONCLUSION

This study showed that feeding of chicks with the pre-starter diets based on corn-soybean meal and rice-soybean meal supplemented with an enzyme complex and probiotic for 0-5 days and 0-10 days positively influence growth performance, carcass yields, villus heights and crypt depths of small intestine segments, except microorganism content of the ileum of broilers on day 42. However, this study also suggests better growth performance, carcass yields and villus heights of small intestine segments of broilers can be achieved if chicks are fed the pre-starter diet based on rice-soybean meal supplemented with enzyme complex and probiotic for 0-5 days. In conclusion, feeding chicks with the pre-starter diet particularly based on rice-soybean meal supplemented with enzyme complex and probiotic for 0-5 days was a more effective treatment in terms of performance especially FI and FCR throughout the experiment, and hot-carcass and cold-carcass yields and villus heights of small intestine segments of broilers on day 42. Nevertheless, there is a need for further nutrigenomic studies to highlight the mechanism of action of the pre-starter diets based on different cereal grains and supplementation with enzyme complex and probiotic on the growth performance, morphological development and microorganism content of small intestine of broilers.

## CONFLICT OF INTEREST

We declare no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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