IJAS Iranian Journal of Applied Animal Science

Effect of Antibiotic, Probiotic and Prebiotic in Diets Containing Barley on Performance, Digestibility, Intestinal Morphology, Blood Parameters and Immunological Response in Broilers

Research Article

M. Mehrabadi[1] and R. Jamshidi[2*]

[1] Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
[2] Department of Biochemistry, Faculty of Veterinary, Semnan University, Semnan, Iran

*Correspondence E-mail: r_jamshidi@semnan.ac.ir

ABSTRACT

This experiment was conducted to determine the effects of replacing corn by 20% barley supplemented with probiotic, prebiotic and antibiotic on performance, immune response, intestinal morphology, carcass development and nutrient digestibility of broilers. Four hundred Ross 308 one day-old broiler chicks were assigned randomly to 5 dietary treatments and were tested for 42 days in a completely randomized design. Six replicates were allotted to each treatment. Experimental dietary treatments included were: T1) diet based on corn and soybean meal (corn control), T2) 20% barley as s replacement for corn without any feed additive and T3, T4 and T5) 20% barley as a replacement for corn with 0.25 g/kg probiotic GalliPro, 1 g/kg prebiotic Fermacto and 15 mg/kg virginiamycin, respectively. There was significant difference within treatments at 42 days of age, where antibiotic treatment had a higher body weight gain than the others. The feed conversion ratio was the lowest in the antibiotic group and was the highest in T2. The use of feed barley with enzymes had considerable effect on the intestinal villi and the villus length was significantly reduced. There was no significant difference in apparent digestibility of nutrients between broiler chicks fed diets with barley or corn. Therefore apparent digestibility of nutrient data showed feed additive in barley-based diet treatments due to improve digestibility in compared to corn treatment. Relative body weight of carcass, breast, tight, liver and abdominal fat and intestinal length at 42 days were recorded. The result indicated that carcass components were not significantly affected by any treatments. The results showed that feeding barley diets with this additives could be a good alternative for corn-based diet.

KEY WORDS barley, broiler, performance, prebiotic, probiotic.

INTRODUCTION

Feed cost is the largest item in poultry production and accounts about for 60 to 75% of the total production cost. Grains are the most important and widely used ingredients in poultry diet. Barley can be included in poultry feed as an energy source. The carbohydrates in barley, however, are not as easily digested as those in corn due to the presence of non-starch polysaccharides mainly beta-glucans.

β-glucans contained in the barley release from cell walls and bind with water in the intestine, resulting in the formation of gels, which increases the viscosity of the intestinal contents (Zielke *et al.* 2017). An increase in viscosity of the digesta adversely affects the digestion and absorption of nutrients. Hooda *et al.* (2011) hypothesized that high viscosity reduces the mixing of intestinal contents and alters the transport properties of the nutrients at the mucosal surface.

Thus, barley β-glucans are considered an anti-nutritional factor that is limiting the nutritional quality of barley (Stein *et al.* 2016).

The negative effects of barley on growth performance of broiler chickens have also been shown to be related to altered intestinal morphology, endogenous enzyme activity, and gut microflora (Jacob and Pescatore, 2012). Broiler chicks fed barley-based diets had increased intestinal viscosity as well as decreased digestive enzyme activities (Yaghobfar and Kalantar, 2017).

Also, the effect on digestive organs has been inconsistent. Brenes *et al.* (1993) reported enlarged pancreases in chicks fed barley-based diets. Also, high intestinal viscosity has been shown to be associated with digestive and health problems. Also, colonization of pathogenic bacteria was become easier by low digesta passage rate (Pan and Yu, 2014). The use of feed enzymes in barley-based diets reduces intestinal viscosity, improving the feeding value of barley. Enzymes also improve the litter quality of poultry raised on barley-based diets (Smits and Anisson, 1996). Antibiotics, probiotic and prebiotic can also increase nutrients digestibility. It has been shown that adding oligosaccharides and lactobacilli to broiler diets increase the activity of β-glucosidase, alpha-galactosidase, aminopeptidase maltase and alkaline phosphatase enzymes. This factor increase nutrients absorption and digestibility.

Esteve-Gracia *et al.* (1997) examined the supplementation of barley-based broilers diets with β-glucanase as well as an antibiotic (flavomycin). Both supplements improved feed intake. It has been reported that probiotics increases feed intake, growth (Allahdo *et al.* 2018), immune responses (Haque *et al.* 2010), the number of *Lactobacilli* in the small intestine and decreases the number of *Escherichia coli* (Xuan *et al.* 2001). Rodríguez *et al.* (2012) compared barley-based broiler diet supplemented with feed enzyme cocktail (xylanase and β-glucanase), a probiotic (*Enterococcus faecium*), a prebiotic (inulin), and a probiotic plus prebiotic combination. The enzyme supplementation improved fat digestion, and the 4 additives had a beneficial effect on the intestinal microflora of broilers. Inulin supplementation reduced the population of *E. coli* in the distal part of the small intestine and addition of the probiotic increased the level of ileal lactic acid bacteria.

Actives probiotic increases digestive utilization of feed and improves growth performances of broilers by stimulating enzymatic activities such as protease and consequently improve digestibility of dry and organic matters in the diets (Ahmed *et al.* 2014).

It may inhibit the proliferation of pathogenic bacteria. Broiler chicken trials in European countries with a total of over 7000 Ross broilers showed that GalliPro increases final body weight.

Bacillus subtilis (DSM 17299) probiotic shows a significant beneficial effect on broiler performance (Lund *et al.* 2005). Virginiamycin is an antibiotic which has been reported to stimulate growth of chick's (Combs *et al.* 1963). Aspergillus meal (Fermacto) is derived from a carefully control fermentation of *Aspergillus oryzae* and has no live cells or spores. Several studies have shown that addition of Fermacto to poultry diets improve performance (Grimes *et al.* 1997). Rodriguez *et al.* (2005) observed that body weight improved by including 0.2% of ferment into the low protein diets in broiler chickens. Also Piray and Kermanshahi (2008) found that adding 15% Fermacto to broiler diets has significant effect on the body weight gain, feed intake and feed conversion ratio.

By using Fermacto, GalliPro and Virginiamycin and thus increasing digestibility we can improve broiler carcass weight. The objective of this study was to determine the effect of adding Fermacto, GalliPro and Virginiamycin to the corn and barley-based diets on performance, immune response, intestinal morphology, ileal digestibility and relative weight of carcass of broilers.

MATERIALS AND METHODS

The experiment was carried out at the Faculty of Agriculture of the University of Tarbiat Modares of Tehran in Iran and according to the National Regulations on Animal Welfare and Institutional Animal Ethics Committee. This experiment was conducted in December 2016.

Animals, management and treatments

Four hundred one day of hatch, commercial Ross 308 female broilers were considered for this experiment. Birds were randomly divided into 5 treatments by 4 replicates and distributed into 40 pens with wood shavings on the floors at the density of 20 birds/pen.

Treatments included: T1) diet based on corn and soybean meal (corn control), T2)- 20% barley as s replacement for corn without any feed additive and T3, T4 and T5) 20% barley as a replacement for corn with 0.25 g/kg probiotic GalliPro, 1 g/kg prebiotic Fermacto and 15 mg/kg virginiamycin, respectively. Each diet that contains barley was fed with Phytase enzyme. Three experimental periods (stater: 1 to 14 d; grower: 14 to 28 d; and finisher: 28 to 42 d) were used in this study. Broilers received the same starter feed (0 to 14 d) based on corn-soybean without any barley.

Experimental diets were fed to chicks from 14 d of age to end of the experiment. The composition of the diets used in this study, which were formulated to contain adequate nutrient levels as defined by the NRC (1994), is presented in Table 1.

Table 1 Composition of the experimental diets

Item	Starter (0-14 d)	Grower (14-28 d)		Finisher (28-42 d)	
Ingredient (%)					
Corn grain	58.32	67.26	45.82	69.46	48.01
Barley garin	-	-	20.00	-	20.00
Soybean meal	36.12	28.11	27.12	25.56	24.57
Oil	1.50	0.57	2.94	0.98	3.35
Dicalcium-phosphate	1.91	1.82	1.73	1.79	1.75
Oyster shell	1.00	0.86	0.92	0.89	0.91
Salt	0.30	0.30	0.29	0.29	0.29
Sodium bicarbonate	0.10	0.10	0.10	0.10	0.10
Vitamin permix[1]	0.25	0.25	0.25	0.25	0.25
Mineral permix[2]	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.24	0.27	0.36	0.26	0.35
L-lysine-HCL	0.01	0.21	0.22	0.17	0.17
Nutrient calculated					
Metabolizable energr (kcal/kg)	2900	2950		3000	
Crude protein (%)	21.0	18.5		17.5	
Lysine (%)	1.21	1.10		1.00	
Arginine	1.38	1.40		1.40	
Methionine (%)	0.54	0.52		0.51	
Calcium (%)	1.00	0.90		0.90	
Available phosphorous (%)	0.47	0.44		0.43	
Sodium (%)	0.15	0.15		0.15	
Methionine + cystin %	0.91	0.87		0.83	

[1] Provided the following nutrients per kilogram of diet: vitamin A: 9000 IU; Cholecalciferol: 2250 IU; vitamin E: 40 mg; vitamin K: 2500 mg; vitamin B_{12}: 10 μg; Riboflavin: 7 mg; Pantothenic acid: 9 mg; Niacin: 44 mg; Folic acid: 0.60 mg; Thiamin: 1 mg; Pyridoxine: 3 mg and Biotin: 0.10 mg.

[2] Provided the following nutrients per kilogram of diet: Manganese: 80 mg; Zinc: 55 mg; Iodine: 1.1 mg; Iron: 22 mg; Copper: 20 mg and selenium: 0.30 mg.

Grains were ground to pass from 2 mm sieve and prepared as mash. Density was approximately 1.041 cm^2/chick. Each pen was equipped with a hanging pan feeder and two nipples waterer. Birds were exposed to 24 h of light for the first 7 d, then to a light: darkness cycle of 22.5 h light: 1.5 h darkness until 42 d of age. Room temperature was maintained at 31 °C for the first 7 d and then was gradually reduced to 22 °C at 42 d of age. Feed and water were available *ad libitum*.

Probiotic and prebiotic preparation
The probiotic product used in the trial was a multi-species GalliPro® (*B. subtilis* 4×10^9 CFU/g) containing *Bifidobacterium animalis subspecies animalis* DSM 16284, *Lactobacillus salivarius subspecies salivarius* DSM 16351, and *Enterococcus faecium* DSM 1913. Prebiotic of Fermacto (mannanoligosaccharide derived from the cell wall of *Saccharomyces cerevisaeyeast*) used was purchased by Biochem Co.

Chemical analysis of feeds
Analysis of dry matter and ash of the diets were measured according to Association Official Analytical Chemists (AOAC, 1990) by oven-drying the samples at 105 °C for 48 h and by ashing in a muffle furnace at 550 °C for 8 h. Ether extract was determined using hexane solvents (AOAC, 1990), and crude protein (CP; N×6.25) was determined

using the Kjeldahl (Kjeltec 2300 Autoanalyzer, Foss Tecator AB, Hoganas, Sweden) method (AOAC, 1990).

Non fibre carbohydrates (NFC) were calculated as: 100 − (% NDF+% CP+% ether extract+% ash). The content of crude protein, crude fat, crude fiber, crude ash, ADF, NDF and β-glucans in barley grain (87% dry matter) used in the present study were 11.67, 1.39, 4.01, 1.97, 19.00, 6.25 and 1.67%, respectively.

Performance parameters
Performance parameters such as, average daily gain (ADG; g/bird), feed conversion ratio (FCR) and feed intake (FI; g/bird) were recorded weekly in the during of experiment from 1 to 42 days of age. Feed intake was determined for each repetition as the difference between the amount of feed supplied and the remaining feed at the end of each experimental period. BW and ADG were calculated as the difference between the final and initial broiler weights. FCR was calculated as the ratio between FI and BW gain during each phase of the experimental period. The feed was inhibit for 5 h before weighing the broiler chicks to ensure the emptying of the digestive tract. Daily mortality was recorded and ADG and FCR were corrected for mortality rate.

Blood parameters and antibody titer
Blood samples were collected at the end of the trial period. For analyses of blood parameters, blood samples were col-

lected by 10 mL Venoject tubes (containing sodium heparin) from the wing veins of 2 broilers per each replicate and were centrifuged at 4 ˚C for 10 min at 3000 × g to separate plasma fluid and frozen at -20 ºC for later chemical analysis (Kuttappan *et al.* 2013). Autoanalyzer (Alcyon 300i Abbott, USA) was used to determine plasma concentration of alanine aminotransferase (ALT; Pars Azmon Company, Tehran, Iran). One bird per replicate was injected under the breast skin with 0.5 mL of a 10% suspension in phosphate buffered saline of sheep red blood cells (SRBC) at the 29th day of age. To determine the systemic antibody response, blood samples were collected from one chick per replicate via the wing vein at the 36th day of age for SRBC. Total immunoglobulin (Ig) and immunoglobulin G (IgG) titers to SRBC were determined by hemagglutination assay; then, immunoglobulin M (IgM) titers to SRBC were calculated as total Ig minus IgG titers. The hemagglutination inhibition titer was expressed as the $\log_2$ of the last dilution of serum that completely inhibited haemagglutination activity (Yogesh *et al.* 2013).

Determination of nutrient digestibility

To determine dry matter, organic matter, energy, protein, and fat apparent ileal digestibility, ileal samples were collected. Titanium oxide (TiO_2) was used as an indigestible marker at 3 g/kg to the final diet (Short *et al.* 1996). Before collecting the feces, in order to bird's adaption, a period of 3 d were considered in 38, 39 and 40th d. Three birds were randomly selected and slaughtered from each replicate at 42 days of age. Their ileal content samples from Meckel's diverticulum to the ileo-cecal junction were collected and placed in numbered nylon bags. These samples were maintained under -20 degree centigrade until basic processes were started. Organic matters were calculated by the differences between dry matter and ash weight. Energy was calculated by Calorimetric bomb, crude protein digestibility calculated by using Kjeldhal method and crude fiber was calculated by Soxhlet method calculation. According to Hashemi *et al.* (2014), apparent ileal digestibility of parameters were estimated using TiO_2 ratios in the diet and ileal content by using the formula:

Apparent ileal digestibility= 100- [(TiO_2 in feed/TiO_2 in ileal content) × (nutrient in ileal content/nutrient in feed) × 100]

Intestinal morphology

To measure the length of the small intestine in centimeters, the small intestine was divided into 3 segments: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the cecum junc-

tion). The contents of the duodenum, jejunum, and ileum (aseptically) were emptied by gentle pressure, and then the length was recorded.

Morphology indices included villus height (from the tip of the villus to the crypt), crypt depth (from the base of the villi to the submucosa), and villus-to-crypt ratio. Two bird per pen was selected to obtain small intestine tissue to measure villus height and crypt depth. The birds were euthanized via cervical dislocation after a 12 h. Fragments of approximately 5 cm in length were obtained from the ileum, between Meckel's diverticulum and the anterior portion of the ileocecal junction. The excised fragments were immersed in a phosphate-buffered formalin solution. Two portions per sample were cut perpendicular to the longitudinal axis of the intestine and embedded in paraffin wax. Transverse sections were cut (3~5 μm). In the morphometric study, images were captured using a light microscope and a system that analyzes computerized images (Bio-Rad Microscience, UK). The height of 10 well oriented villi and their associated crypts were measured from each replicate per segment. The mean was obtained from these values. Villus height (mm) was measured from the tip of the villus to the villus crypt junction and crypt depth was measured from the base upward to the region of transition between the crypt and villus height: crypt depth ratio was then calculated. The intestinal morphology was done according to the method of Eftekhari *et al.* (2015).

Carcass characteristics

To evaluate the carcass yield of broilers at 42 days of age, two poultry per experimental unit, of average weight (10% above or below average) were kept fasted for 6 hours and slaughtered. After bleeding, birds were plucked and eviscerated. The hot carcass was weighed and sent to evaluation of the cuts yields (carcass, breast, thigh, liver and abdominal fat) relative to the weight of the eviscerated carcass.

Statistical analysis

Data were analyzed based on completely randomized design with 4 replications for each level of the treatments. Comparison of the means of non-additive versus additives groups was tested for significance using Duncan's multiple range test of SAS (2002) using the model:

$$y_{ij} = \mu + T_i + e_{ij}$$

Where:

y_{ij}: response variable.

μ: overall mean.

T_i: effect of the probiotic, prebiotic and antibiotic.

e_{ij}: residual error.

Differences among means were considered statistically significant at P < 0.05.

RESULTS AND DISCUSSION

Broiler performance

Results of different experimental treatments on average daily gain (ADG), feed intake (FI) and feed conservation ratio (FCR) for 3 periods of 14-28, 28-42 and 14-42 d are presented in Table 2. There were no significant difference in feed intake during starter (14-28 days), grower (28-42 days) and overall period (14-42 days) of chickens fed diets with treatments among the groups, although the feed intake of broilers in replacing corn with 20% barley (barley control) was higher than those of treatments probiotic, prebiotic and control (P>0.05). There was no significant difference among treatments in average daily gain in 14-28 and 28-42 d, but the average daily gain in 14-42 d of broilers in virginiamycin treatment was significantly (P<0.05) higher than those of barley control treatment (1819.16 *vs*. 1636.00 g). Between 14-42 d, FCR significantly increased (P<0.05) in broilers fed diets containing 20% barley compared to other treatments, but no significant differences (P>0.05) were observed between FCR in 14-28 and 28-42 d.

Nutrient digestibility

Results of different experimental treatments on nutrient ileal digestibility are presented in Table 3. Although between different experimental group no significant differences (P>0.05) were noted for dry mater digestibility at 42 days, but numerically, experimental groups fed with diet contain 20% barley, had the lowest (83.75%) and those fed with corn-based diet had the highest (85.37%) digestibility. There were no significant (P>0.05) effects of the diets on the digestibility of organic matter, energy, protein and fat of broilers.

Carcass yield

Effects of different experimental treatment on percentage of carcass, breasts, thighs, liver and abdominal fat per live body mass are presented in Table 4. No significant differences were noted for percentage of carcasses, breasts and thighs. Numerically, antibiotic treatment had the highest level of this three item and corn diet had the lowest level. Experimental treatments did not cause any significant effect on relative weights of liver and abdominal fat.

Intestinal morphology

T Results of the intestinal morphology of broiler chickens at 42 day of age are presented in Table 5. The results showed dietary supplementation including of probiotic, prebiotic and antibiotic had no significant effect on the intestine length included duodenum, jejunum, ileum and total length during the entire of experimental period (P>0.05). The villus height, crypt depth, and height:crypt depth ratio of small intestine from treatments are shown in Table 6.

The height, crypt depth and height:crypt of jejunum and ileum and crypt depth and height: crypt of deodenum were not markedly different between reatments (P>0.05). In contrast, the villus height of deodenum was significantly (P<0.05) higher in antibiotic than in barley control treatment (1.55 *vs*. 1.30).

Blood parameters and immunological response

As presented in Table 7, dietary supplementation of probiotic had significant effect on antibody titer included IgG and IgM (P<0.05). Dietary supplementation of probiotic significantly increased the primary immune response to SRBC injection; serum IgG and IgM concentration significantly increase in response to the SRBC inoculation. The use of probiotic increased the immune system (IgG and IgM) compared to barley treatment (2.9 *vs*. 1.5 for IgG and 6.0 *vs*. 4.4 for IgM). On the other hand, the serum concentration of alanine aminotransferase (liver enzyme) was not affected by the adding probiotic, pribiotic and antibiotic to the broiler diet at 42 day of age (Table 7).

Broiler performance

A number of studies with broiler chickens have reported that feeding of antibiotic, probiotic and prebiotic improved BW gain and feed conversion ratio (Khan and Iqbal, 2016; Tayeri *et al*. 2018: Nosrati *et al*. 2017). However, some studies have shown no positive effects on growth performance for these edible additives (Tung and Herzenberg, 2007; Murshed and Abudabos, 2015; Abudabos *et al*. 2017). Khaksefidi and Rahimi (2005) reported that feeding of multi-species probiotics increased BW gain and feed conversion rate in finisher period.

Consistent improvement in functional parameters in broilers fed probiotic and prebiotic has also reported by Huang *et al*. (2004).

Khan and Iqbal (2016) reported that feeding of probiotic resulted in a higher villus height, indicating that probiotics was potentially able to enhance nutrients absorption and thereby improve growth performance parameters in broiler chickens. In this investigation, replacing corn with 20% barley (barley control) decreased average daily gain and increased FCR in broilers during the 14-42 d, but feeding probiotic, antibiotic and prebiotic improved average daily gain and FCR in the finisher period.

The results reported by Toghyani *et al*. (2011) agree with our findings, they reported that application of probiotic, prebiotic and antibiotic in diet increased average daily gain of broiler chicks at 42 d.

Table 2 Effect of different dietary treatments on feed intake (FI), average daily gain (ADG) and feed conversion ration (FCR) in broiler chickens

Treatments	FI (g)			ADG (g)			FCR		
	14-28 d	28-42 d	14-42 d	14-28 d	28-42 d	14-42 d	14-28 d	28-42 d	14-42 d
Corn and soybean based diet (corn control)	986	1638.6	3125.90	688.30	691.50	1731.01[ab]	1.43	2.47	1.81[b]
Replacing corn with barley at 20% (barley control (BC))	1128.4	1637	3224.70	610.96	691.47	1636.00[b]	1.83	2.29	1.96[a]
BC + GalliPro	965.9	1516.4	2994.70	697.83	697.66	1734.21[ab]	1.40	2.20	1.71[b]
BC + Fermecto	1029.4	1524.8	3101.05	668.67	743.00	1754.78[ab]	1.55	2.04	1.76[b]
BC + virginiamycin	966	1673	3172.20	672.68	791.91	1819.16[a]	1.50	2.12	1.74[b]
SEM	36.89	39.29	35.09	13.80	18.72	20.13	0.062	0.071	0.026
P-value	0.545	0.562	0.229	0.324	0.374	0.050	0.187	0.387	0.012

SEM: standard error of the means.

Table 3 Effect of different dietary treatments on apparent ileal nutrient digestibility (%) in broiler chickens

Treatments	Parameters				
	Dry matter	Organic matter	Energy	Protein	Fat
Corn and soybean based diet (corn control)	85.37	75.20	75.00	75.50	62.25
Replacing corn with barley at 20% (barley control (BC))	83.75	75.74	73.00	73.50	64.00
BC + GalliPro	84.00	75.00	75.00	74.50	65.00
BC + Fermecto	84.37	74.25	84.75	73.25	63.75
BC + virginiamycin	84.00	75.25	74.75	74.25	62.75
SEM	0.43	0.46	0.33	0.54	0.53
P-value	0.81	0.97	0.29	0.74	0.62

SEM: standard error of the means.

Table 4 Effect of different dietary treatments on relative weight percentage of abdominal fat and carcass

Treatments	Parameters (%)				
	Carcass	Breast	Thigh	Liver	Abdominal fat
Corn and soybean based diet (corn control)	67.34	32.90	28.43	1.90	1.75
Replacing corn with barley at 20% (barley control (BC))	70.46	35.18	29.28	1.99	1.67
BC + GalliPro	70.32	34.17	30.14	1.85	1.80
BC + Fermecto	71.50	35.12	30.38	1.76	1.68
BC + virginiamycin	73.39	35.87	31.51	1.70	1.83
SEM	0.79	0.53	0.80	0.04	0.02
P-value	0.18	0.49	0.83	0.15	0.33

SEM: standard error of the means.

Table 5 Effect of different dietary treatments on the length of different part of intestine

Treatments	Parameters (cm)			
	Deodenum	Jejunm	İleum	Total
Corn and soybean based diet (corn control)	33.05	81.30	82.12	196.47
Replacing corn with barley at 20% (barley control (BC))	36.07	85.72	84.72	206.25
BC + GalliPro	33.91	79.99	85.30	199.21
BC + Fermecto	34.58	84.33	85.92	204.95
BC + virginiamycin	34.00	75.24	84.72	186.53
SEM	0.69	1.43	1.20	2.49
P-value	0.76	0.15	0.12	0.06

SEM: standard error of the means.

In addition, it has been reported that diet supplementation with probiotic increased final body weight gain and improved FCR at week 6 of age in broiler chicks (Panda *et al.* 1999). Although, Midilli *et al.* (2008) were unsuccessful to observe any improvement on productive traits of broilers fed prebiotic and prebiotic supplemented diets.

Nutrient digestibility

In the present study, no differences (P>0.05) in apparent ileal digestibility of dry or organic matter, energy, protein and fat were observed between treatments at 42 d. The results reported by Arena *et al.* (2014) also agree with our findings, these scientists observed no differences in nutrient digestibility of broiler chicks fed barley-based diet supplemented with probiotic. The barley-based diet which was treated with enzyme, probiotic or prebiotic showed the same results as the corn diet, indicating that the efficiency of dietary utilization increased in broiler chicks fed these regimens. Non-starch polysaccharides in barley had the highest effect on fat digestibility.

502

Table 6 Effect of different dietary treatments on the height, crypt depth and height:crypt of parts of small intestine

Treatments	Deodenum (mm)			Jujnum (mm)			Ileum (mm)		
	Height	Depth	H/D	Height	Depth	H/D	Height	Depth	H/D
Corn and soybean based diet (corn control)	1.42[ab]	0.287	0.835	0.925	0.247	3.78	0.707	0.205	3.45
Replacing corn with barley at 20% (barley control (BC))	1.30[b]	0.305	0.874	0.830	0.272	3.10	0.712	0.207	3.44
BC + GalliPro	1.38[b]	0.270	6.07	0.825	0.232	3.61	0.687	0.205	3.77
BC + Fermecto	1.42[ab]	0.285	5.78	0.887	0.250	3.60	0.762	0.217	3.22
BC + virginiamycin	1.55[a]	0.285	5.74	0.867	0.270	3.21	0.725	0.215	3.40
SEM	0.027	0.004	0.191	0.016	0.007	0.119	0.009	0.005	0.093
P-value	0.056	0.115	0.355	0.261	0.467	0.340	0.154	0.933	0.493

SEM: standard error of the means.

Table 7 Effect of different dietary treatments on ALT and Immunological response in broiler chickens

Treatments	Parameters		
	IgG (mg/dL)	IgM (mg/dL)	ALT (IU/L)
Corn and soybean based diet (corn control)	2.30[ab]	4.86[bc]	4.53
Replacing corn with barley at 20% (barley control (BC))	1.50[c]	4.40[c]	4.76
BC + GalliPro	2.90[a]	6.00[a]	4.50
BC + Fermecto	2.33[ab]	4.86[bc]	4.33
BC + virginiamycin	2.16[b]	5.36[ab]	5.03
SEM	0.08	0.75	0.12
P-value	0.03	0.04	0.13

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Using antibiotic in barley diets had the lowest effect on fat digestibility in comparison with other additives and also with barley control diet. Low fat digestibility in groups supplemented with antibiotic could be due to this fact that probiotics can be destroyed by antibiotic. Thus, it prevents fat emulsfication and also digestion by increasing fat DE-conjugation and inhibition of pancreatic enzymes. Enzyme presence in barley-based diets, break down the polysaccharides linkage and makes the nutrients available for birds. Antibiotics destroyed competitive harmful microorganisms and probiotic placed useful microorganism in digestive tract.

Thus, all of this additives, increase absorption and body weight. Barley polysaccharides significantly decrease nitrogen apparent digestibility (Smits et al. 1997). Moharrery and Mohammadpour (2005) indicated that enzyme supplementation decrease uric acid and increase protein digestibility and availability.

Huang et al. (2005) indicated that diet supplementation with chitosan oligosaccharides (COS) increase ileal nutrients digestibility and performance in broilers at 21 and 42 days of age. Increase of apparent ileal digestibility by use of prebiotics, in one hand is due to decrease of pathogen bacteria such as E. coli and Salmonella typhimurium (LeMieux et al. 2003; Wang et al. 2003) and increasing useful bacteria such as lactobacilli (Hou and Gou, 2001) and in other hand is due to improve nutrients absorption by decreasing the competition of host and harmful pathogens (Stanley et al. 2000).

Carcass yield

Although Novak et al. (2011) reported increases in percentage of carcass for probiotic supllementation goups, but most of research did not indicate a significant effect for antibiotic, prebiotic and probiotic on carcass (Cengiz et al. 2015; Pelicano et al. 2005; Denli et al. 2003). Relative weights of abdominal fat and liver were not significantly influenced by dietary treatments. But numerically the highest relative weight of liver was fror T2 group. In general, each factor that increase organ activity, more than threshold, can increase its weight by hypertrophic interactions.

Whenever gut track digesta has high viscosity, mixing feed and enzymes and bile acids were not properly done. Thus, we need more bile acids for fat emulsification and this result might because of barley ration high viscosity (Viveros et al. 1994).

Researchers indicated that liver weight in bird fed rye diets were relatively higher than those fed with corn diets (Lazaro et al. 2004). In contrast Bernes et al. (1993) noted that enzyme supplementation to barley diets decrease relative weight of proventriculus, pancreas, liver, duodenum, jejunum and caecum.

Nahas and Lefrancois (2001) found that, by feeding barley to broilers, liver weight increased. Highest abdominal fat in this experiment is related to antibiotic treatment. This might be due to reduction of immune response (Humphrey et al. 2002) and increase of fat retention. A possible reason for the differences between findings of different researchers may be related to the doses or type of probiotic and pre-

biotic, kind of animal, strains of microorganism used and composition of diets.

Intestinal morphology

Although, no differences in intestine length were found between treatments (Table 4), but intestine length was higher in birds received barley and enzyme and was lower in treatments received antibiotic in compared with the other groups. Decrease in intestine length might be due to increase of nutrient availability or decrease of harmful bacterial activity and effects (Visek, 1978). These results correspond with the results of Miles *et al.* (2006) which indicated that the use of antibiotic as promotor decrease intestine length. Visek (1978) indicated that, growth promotor antibiotics decrease intestine weight by decreasing its length and thickness of its wall. Thickness of lamina propria layers increase by feeding growth promoter antibiotics. Goldin (1998) reported that with adding the growth promoter antibiotics to chicks diets, thickness of this layer was similar with new born chicks. Feeding fiber-rich diets, increase length of intestine by increasing the digesta viscosity. Increase of intestine length might be due to the increase of intestine activity in terms of speeding the passage of intestinal contents (Rubio *et al.* 1990). Feeding barley diets with enzyme can partially decrease intestinal tract length. Farhoomand and Dadvend (2007) reported that feeding probiotics decrease intestine length by improving nutrient efficiency, availability, digestibility and absorption. Wang *et al.* (2003) reported that feeding oligosaccharides increase intestine length especially in female broilers. Also, Denli *et al.* (2003) found that feeding probiotic, organic acids and antibiotics, improved intestinal tract length, although this effect were not significant.

Chickens fed diets contained antibiotic had the longer villi, higher than those replacing corn with 20% barley supplemented in diet (Table 6). The results obtained from this study showed that dietary supplementation of virginimycine significantly improved intestinal growth and development. The results of another experiment showed that intestinal morphometric characteristics such as the villi height and crypt depth following the incorporation of antibiotic in the diet have improved (Garcia *et al.* 2007). The length and diameter of the intestinal villi are morphological indices for absorptive activity of intestinal epithelial cells, so each increment leading to an increase in the absorption capacity of the intestine (Markovic *et al.* 2009). Therefore, this kind of morphological changes induced in the intestine can indicate the effect of growth promoters on changes in the level of intestinal absorption and hence the change in the performance of broiler chickens (Oliveira *et al.* 2008).

Blood parameters and immunological response

In our study, maternal antibody titres of the chicks was differ significantly (P<0.05). This is in agreement with Racedo *et al.* (2006) in which it was found that immune response in broiler chicks was affected by the supplementation of probiotic in diet. Comparison of antibody titres of probiotic treatment and barley control suggests that the probiotics may have had an immunomodulatory role. Oral administration of *Lactobacillus* strains is reported to significantly enhance the IgG response (Maassen *et al.* 2000), and administration of lactic acid bacteria produced higher levels of anti-pneumococcal serum IgG and bronchoalveolar lavage IgA in mice (Racedo *et al.* 2006). The exact mechanisms of immunomodulation by probiotics have not been fully explained but they may stimulate different subsets of immune system cells (Maassen *et al.* 2000; Bal *et al.* 2004).

Oral administration of probiotics can significantly affect both the systemic and mucosa-associated immune responses, resulting in disease prevention (Dallout *et al.* 2003). We observed no significant difference of treatments on ALT in among the treatments, but a numerical increase was observed with treatments inclusion with the highest ALT concentration occurring in the replacing corn with 20% barley + virginiamycin. These results did not support the previous findings that showed that probiotic a decrease in ALT (Nabavi *et al.* 2014).

CONCLUSION

In summary, the results of this experiment indicated that feeding corn diets and 20% barley with 0.25 g/kg GalliPro as probiotic or 1.00 g/kg Fermacto as prebiotic or 15 mg/kg virginiamycin as antibiotic can improve performance, immune responses, blood parametes, digestibility and carcass developpment and these diets have similar effect with corn-based diet. Therefore, it would be concluded that the barley based-diet plus foregoing additives have to be applied as an effective substitute for corn-based diet.

REFERENCES

Abudabos A.M., Alyemni A.H., Dafalla Y.M. and Al-Owaimer A.N. (2017). Effect of organic acid blend and *Bacillus subtilis* alone or in combination on growth traits, blood biochem-ical and antioxidant status in broiler exposed to clostrid-ium perfringens challenge during the finisher phase. *J. Anim. Plant Sci.* **27,** 1101-1107.

Ahmed S.T., Islam M., Mun H.S., Sim H.J., Kim Y.J. and Yang C.J. (2014). Effects of *Bacillus amyloliquefaciens* as a probiotic strain on growth performance, cecal microflora, and fecal noxious gas emissions of broiler chickens. *Poult. Sci.* **9,** 1963-1971.

Allahdo P., Ghodraty J. and Zarghi H. (2018). Effect of probiotic and vinegar on growth performance, meat yields, immune responses, and small intestine morphology of broiler chickens. *Italian J. Anim. Sci.* **17(3),** 675-685.

AOAC. (1990). Official Methods of Analysis. Vol. I. 15th Ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Arena M.P., Caggianiello G., Fiocco D., Russo P., Torelli M., Spano G. and Capozzi V. (2014). Barley β-glucans-containing food enhances probiotic performances of beneficial bacteria. *Int. J. Mol. Sci.* **15(2),** 3025-3039.

Bal A.P., Ouyang Q., Zhang W., Wang C.H. and Li S.F. (2004). Probiotics inhibit TNF-a-induced interleukin-8-secreation of HT29 cells. *World J. Gastroenterol.* **10,** 455-457.

Bernes A., Smit M., Guenter W. and Marquardt R.R. (1993). Effect of enzyme supplementation on the performance and digestive tract size of broiler chickens fed wheat and barley-based diet. *Poult. Sci.* **72,** 1731-1739.

Cengiz Ö., Köksal B.H., Tatlı O., Sevim O., Ahsan U. and Üner A.G. (2015). Effect of dietary probiotic and high stocking density on the performance, carcass yield, gut microflora, and stress indicators of broilers. *Poul. Sci.* **94,** 2395-2403.

Combs G.F. and Brossard E.H. (1963). Comparison of growth response of chicks to virginiamycin and other antibiotics. *Poult. Sci.* **42,** 681-685.

Dallout R.A., Lillehoj H.S., Shellem T.A. and Doerr J.A. (2003). Enhanced mucosal immunity against Eimeria acervulina in broilers fed a *Lactobacillus* -based probiotic. *Poult. Sci.* **82,** 62-66.

Denli M., Okan F. and Celic K. (2003). Effect of dietary probiotic, organic acid and antibiotic supplementation to diets on broiler performance and carcass yield. *Pakistan J. Nutr.* **2,** 89-91.

Eftekhari A., Rezaeipour V. and Abdullahpour R. (2015). Effects of acidified drinking water on performance, carcass, immune response, jejunum morphology, and microbiota activity of broiler chickens fed diets containing graded levels of threonine. *Livest. Sci.* **180,** 158-163.

Esteve-Garcia E., Brufau J., Pérez-Vendrell A., Miquel A. and Duven K. (1997). Bioefficacy of enzyme preparations containing β-glucanase and xylanase activities in broiler diets based on barley or wheat, in combination with flavomycin. *Poult. Sci.* **76,** 1728-1737.

Farhoomand P. and Dadvend A. (2007). Carcass weight, growth performance and intestinal organs size of broilers fed graded levels of *Saccharomycese cervicia* supplementation diets. *Pakistan J. Biol. Sci.* **10(11),** 1870-1874.

Garcia V., Catala Gregori P., Hernandez F., Megias M.D. and Madrid J. (2007). Effect of formic acid and plant extracts on growth, nutrient digestibility, intestine mucosa morphology, and meat yield of broilers. *J. Appl. Poult. Res.* **16,** 555-562.

Goldin B.R. (1998). Health benefits of probiotics. *Br. J. Nutr.* **80,** 203-207.

Grimes J.L., Maurice D.V., Lightsey S.F. and Lopez G.H. (1997). The effect of dietary fermato on layer hen performance. *J. Appl. Poult. Res.* **6,** 399-403.

Hashemi S.R., Loh T.C., Foo H.L. and Zulkifli M. (2014). Intestinal histomorphology changes and serum biochemistry

responses of broiler chickens fed herbal plant (*Euphorbia hirta*) and mix of acidifier. *Iranian J. Appl. Anim. Sci.* **4,** 95-103.

Haque M.N., Islam K.M., Akbar M.A., Chowdhury R., Khatun M., Karim M.R. and Kemppainen B.W. (2010). Effect of dietary citric acid, flavomycin and their combination on the performance, tibia ash and immune status of broiler. *Canadian J. Anim. Sci.* **90,** 57-63.

Hooda S., Metzler-Zebeli B.U., Vasanthan T. and Zijlstra R.T. (2011). Effects of viscosity and fermentability of dietary fibre on nutrient digestibility and digesta characteristics in ileal-cannulated grower pigs. *Br. J. Nutr.* **106,** 664-674.

Hou Q.L. and Gao Q.S. (2001). Chitosan and Medicine. Shanghai Science Technology Press, Shanghai, China. *Immunology.* **12(12),** 1387-1392.

Huang M.K., Choi Y.G., Houde R., Lee J.W., Lee B. and Zhao X. (2004). Effects of Lactobacilli and Acidophilic fungus on the production performance and immune responses in broiler chickens. *Poult. Sci.* **83,** 788-795.

Huang R.L., Yin Y.L., Wu G.Y., Zhang T.J., Li L.L., Li M.X., Tang Z.R., Zhang J., Wang B., He J.H. and Nie X.Z. (2005). Effect of dietary oligochitosan supplementation on ileal nutrient digestibility and performance in broilers. *Poult. Sci.* **84,** 1383-1388.

Humphrey B.D., Koutsos E.A. and Klasing K.C. (2002). Requirements and priorities of the immune system for nutrients. Pp. 69-77 in Biotechnology in the Feed and Food Industry. K.A. Jacques and T.P. Lyons, Eds. Nottingham University Press, Nottingham, United Kingdom.

Jacob J.P. and Pescatore A.G. (2012). Using barley in poultry diets: A review. *J. Appl. Poult. Res.* **21,** 915-940.

Khaksefidi A. and Rahimi S. (2005). Effect of probiotic inclusion in the diet of broiler chickens on performance, feed efficiency and carcass quality. *Asian-Australasian J. Anim. Sci.* **18,** 1153-1156.

Khan S.H. and Iqbal J. (2016). Recent advances in the role of organic acids in poultry nutrition. *J. Appl. Anim. Res.* **44,** 359-369.

Kuttappan V.A., Huff G.R., Huff W.E., Hargis B.M., Apple J.K., Coon C. and Owens C.M. (2013). Comparison of hematologic and serologic profiles of broiler birds with normal and severe degrees of white striping in breast fillets. *Poult. Sci.* **92(2),** 339-345.

Lazaro R., Latorre M.A., Medel P., Gracia M. and Mateos G.G. (2004). Feeding regimen and enzyme supplementation to Rye-based diets for broilers. *Poul. Sci.* **83,** 152-160.

LeMieux F.M., Southern L.L. and Bidner T.D. (2003). Effects of mannan oligosaccharides on growth performance of weanling pigs. *J. Animl. Sci.* **81,** 2462-2487.

Lund B., Hansen S. and Kürti P. (2005). Efficacy of GalliPro - a microbial feed additive for broilers. Pp. 263-265 in Proc. 15th European Symp. Poult. Nutr., Belatonfüred, Hungary.

Maassen C.B., van Holten-Neelen C., Balk F., den Bak-Glashouwer M.J., Leer R.J., Laman J.D., Boersma W.J. and Claassen E. (2000). Strain-dependent induction of cytokine profiles in the gut by orally administered *Lactobacillus* strains. *Vaccine.* **18,** 2613-2623.

505

Markovic R., Sefer D., Krstic M. and Petrujki B. (2009). Effect of dif-ferent growth promoters on broiler performance and gut morphology. *Arch. Med. Vet.* **41,** 163-169.

Midilli M., Alp M., Kocabagli N., Muglali Ö.H., Turan N., Yilmaz H. and Çakir S. (2008). Effects of dietary probiotic and prebiotic supplementation on growth performance and serum IgG concentration of broilers. *South African J. Anim. Sci.* **38,** 21-27.

Miles R.D., Butcher G.D., Henry P.R. and Littell R.C. (2006). Effect of antibiotic growth promoters on broiler performance, intestinal growth paramerers, and quantitative morphology. *Poult. Sci.* **85,** 476-486.

Moharrery A. and Mohammadpour A.A. (2005). Effect of diets containing different qualities of barley on growth performance and serum amylase and intestinal villus morphology. *Int. Poult. Sci.* **4(8),** 549-556.

Murshed M.A. and Abudabos A.M. (2015). Effects of dietary inclusion of probiotic, prebiotic and symbiotic on growth perform-ance of broiler chickens. *Brazilian J. Poult. Sci.* **117,** 99-104.

Nabavi S., Rafraf M., Somi M.H., Homayouni-Rad A. and Asghari Jafarabadi M. (2014). Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. *J. Dairy Sci.* **97(12),** 7386-7393.

Nahas J. and Lefrancois R. (2001). Effects of feeding locally grown whole barley with or without enzyme addition and whole wheat on broiler performance and carcass traits. *Poult. Sci.* **80,** 195-202.

Nosrati M., Javandel F., Camacho L.M., Khusro A., Cipriano M., Seidavi A.R. and Salem A.Z.M. (2017). The effects of antibiotic, probiotic, organic acid, vitamin C, and Echinacea purpurea extract on performance, carcass characteristics, blood chemistry, microbiota, and immunity of broiler chickens. *J. Appl. Poult. Res.* **26(2),** 295-306.

Novak R., Matijašić B., Bogovič Terčič D., Červek M., Gorjanc G., Holcman A., Levart A. and Rogelj I. (2011). Effects of two probiotic additives containing *Bacillus* spores on carcass characteristics, blood lipids and cecal volatile fatty acids in meat type chickens *J. Anim. Physiol. Anim. Nutr.* **95,** 424-433.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, D.C., USA.

Oliveira M.C., Rodrigues E.A., Marques R.H., Gravena R.A., Guandolini G.C. and Moraes V.M.B. (2008). Performance and morphology of intestinal mucosa of broilers fed mannano-ligosaccharides and enzymes. *Arq. Bras Med. Vet. Zootec.* **60,** 442-448.

Pan D. and Yu Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet. *Gut Microbes.* **5,** 108-119.

Panda A.K., Ramarao S.V., Reddy M.R. and Praharaj N.K. (1999). Effect of dietary inclusion of probiotic on growth, carcass traits and immune response in broilers. *Indian J. Poult. Sci.* **34,** 343-346.

Pelicano E.R.L., Souza P.A., Souza H.B.A., Figueiredo D.F., Boiago M.M., Carvalho S.R. and Bordon V.F. (2005). Intestinal mucosa development in broiler chickens fed natural growth promoters. *Brazilian J. Poult. Sci.* **7(4),** 221-229.

Piray A.H. and Kermanshahi H. (2008). Effect of different supplementation of Aspergillus meal prebiotic (Fermacto) on efficiency serum lipids and immunity responses of broiler chickens. *J. Biol. Sci.* **8(4),** 818-821.

Racedo S., Villena J., Medina M., Aguero G., Rodríguez V. and Alvarez S. (2006). *Lactobacillus casei* administration reduces lung injuries in a *Streptococcus* pneumoniae infection in mice. *Microbes Infect.* **8,** 2359-2366.

Rodriguez M.L., Rebolé A., Velasco S., Ortiz L.T., Treviño J. and Alzueta C. (2012). Wheat and barley based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *J. Sci. Food Agric.* **92,** 184-190.

Rodriguez T.A., Sater C., Higgins C.E., Wolfenden A.D., Bielke L.R., Pixley C.M., Sutoon L., Tellez G. and Hargis B.M. (2005). Effect of *Aspergillus* meal prebiotic (Fermacto) on performance of broiler chickens in the starter phase and fed low protein diets. *J. Appl. Poult. Res.* **14,** 665-669.

Rubio L.A., Brenes A. and Castano M. (1990). The utilization of raw and autoclaved faba beans (*Vicia faba*) and faba bean fractions in diets for growing broiler chickens. *British J. Nutr.* **63,** 419-433.

SAS Institute. (2002). SAS®/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC. USA.

Short F.J., Gorten P., Wiseman J. and Boorman K.N. (1996). Determination of titanium dioxide added as an insert marker in chicken digestibility studies. *Anim. Feed Sci. Technol.* **59,** 215-221.

Smits C.H.M. and Anisson G. (1996). Non-starch plant polysacharidesin broiler nutrition- towards a physiologically valid approach to their determination. *World's Poult. Sci.* **52,** 203-221.

Smits C.H.M., Veldman A., Verstegen M.W.A. and Beynen A.C. (1997). Dietary carboxy methyl cellulose with high instead of low viscosity reduces macronutrient digestion in broiler chickens. *J. Nutr.* **127,** 483-487.

Stanley V.G., Brown C. and Sefton A.E. (2000). Comparative evaluation of yeastculture, mannan-oligosaccharide and antibiotic on performance of turkeys. *Poult. Sci.* **79(1),** 186-195.

Stein H.H., Lagos L.V. and Casas G.A. (2016). Nutritional value of feed ingredients of plant origin fed to pigs. *Anim. Feed Sci. Technol.* **218,** 33-69.

Tayeri V., Seidavi A., Asadpour L. and Phillips C.J.C. (2018). A comparison of the effects of antibiotics, probiotics, synbiotics and prebiotics on the performance and carcass characteristics of broilers. *Vet. Res. Commun.* **42(3),** 195-207.

Toghyani M., Toghyani M. and Tabeidian A. (2011). Effect of probiotic and prebiotic as antibiotic growth promoter substitutions on productive and carcass traits of broiler chicks. Pp. 82-86 in Int. Conf. Food Eng. Biotechnol., Singapoore.

Tung J. and Herzenberg L. (2007). Unraveling B-1 progenitors. *Curr. Opin. Immunol.* **19,** 150-155.

Visek W.J. (1978). The mode of growth promotion by antibiotics. *J. Anim. Sci.* **46,** 1447-1469.

Viveros A., Brenes A., Pizarro M. and Castano M. (1994). Effect of enzyme supplementation of a diet based on barley and

autoclave treatment, on apparent digestibility, growth performance, and gut morphology of broilers. *Animl. Feed Sci. Technol.* **48,** 237-251.

Wang X.W., Du Y.G., Bai X.F. and Li S.G. (2003). The effect of oligochitosan on broiler gut flora, microvilli density, immune function and growth performance. *Acta Zoonutr. Sin.* **15,** 32-45.

Xuan Z.N., Kim J.D., Heo K.N., Jung H.J., Lee J.H., Han Y.K., Kim Y.Y. and Han I.N.K. (2001). Study on the development of a probiotics complex for weaned pigs. *Asian–Australasian J. Anim. Sci.* **14,** 1425-1428.

Yaghobfar A. and Kalantr M. (2017). Effect of non–starch polysaccharide (NSP) of wheat and barley supplemented with exogenous enzyme blend on growth performance, gut microbial, pancreatic enzyme activities, expression of glucose transporter (*SGLT1*) and mucin producer (*MUC2*) genes of broiler chickens. *Brazilian J. Poult. Sci.* **19(4),** 629-638.

Yogesh K., Chandra D., Shrivastava H.P., Mandal A.B., Ashutosh W. and Indira S. (2013). Growth performance, carcass yield, and immune competence of broiler chickens as influenced by dietary supplemental zinc sources and levels. *Agric. Res.* **2(3),** 270-274.

Zielke C., Kosik O., Ainalem G., Lovegrove A., Stradner A. and Nilsson A. (2017). Characterization of cereal β-glucan extracts from oat and barley and quantification of proteinaceous matter. *Infect. Immun.* **72,** 4159-4171.