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

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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 a 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 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.

\[ \beta \text{-glucans} \] 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 (Yaghobifar 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). Rodriguez 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 (starter: 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.
Grains were ground to pass from 2 mm sieve and prepared as mash. Density was approximately 1.041 cm³/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 4×10⁹ 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 cerevisiae) 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 (Kjeltac 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 °C for later chemical analysis (Kuttappan et al. 2013). Autoanalyzer (Alycyon 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 log2 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 (TiO2) 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 TiO2 ratios in the diet and ileal content by using the formula:

\[
\text{Apparent ileal digestibility} = 100 - \left[ \left( \frac{\text{TiO}_2 \text{ in feed}}{\text{TiO}_2 \text{ in ileal content}} \right) \times \left( \frac{\text{nutrient in ileal content}}{\text{nutrient in feed}} \right) \times 100 \right]
\]

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 junction). 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.
- \(\mu\): 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 conversion 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 dry matter digestibility at 42 days, but the average daily gain in 14-42 d was significantly higher than that of barley control treatment ($1819.16 \, \text{vs.} \, 1636.00 \, \text{g}$). 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 there were no significant differences ($P>0.05$) were noted for dry matter 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 treatments ($P>0.05$). 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, prebiotic and antibiotic to the broiler diet at 42 day of age (Table 7).
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.
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 emulsification 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).

Carass yield

Although Novak et al. (2011) reported increases in percentage of carass for probiotic supplementation goups, but most of research did not indicate a significant effect for antibiotic, prebiotic and probiotic on carass (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 for 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-

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Deodenum (mm)</th>
<th>Jujnum (mm)</th>
<th>Ileum (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Height</td>
<td>Depth</td>
<td>H/D</td>
</tr>
<tr>
<td>Corn and soybean based diet (corn control)</td>
<td>1.42a</td>
<td>0.287</td>
<td>0.835</td>
</tr>
<tr>
<td>Replacing corn with barley at 20% (barley control (BC))</td>
<td>1.30b</td>
<td>0.305</td>
<td>0.874</td>
</tr>
<tr>
<td>BC + GalliPro</td>
<td>1.38b</td>
<td>0.270</td>
<td>6.07</td>
</tr>
<tr>
<td>BC + Fermecto</td>
<td>1.42ab</td>
<td>0.285</td>
<td>5.78</td>
</tr>
<tr>
<td>BC + virginiamicin</td>
<td>1.55c</td>
<td>0.285</td>
<td>5.74</td>
</tr>
<tr>
<td>SEM</td>
<td>0.027</td>
<td>0.004</td>
<td>0.191</td>
</tr>
<tr>
<td>P-value</td>
<td>0.056</td>
<td>0.115</td>
<td>0.355</td>
</tr>
</tbody>
</table>

SEM: standard error of the means.

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG (mg/dL)</td>
</tr>
<tr>
<td>Corn and soybean based diet (corn control)</td>
<td>2.30a</td>
</tr>
<tr>
<td>Replacing corn with barley at 20% (barley control (BC))</td>
<td>1.50c</td>
</tr>
<tr>
<td>BC + GalliPro</td>
<td>2.90c</td>
</tr>
<tr>
<td>BC + Fermecto</td>
<td>2.33ab</td>
</tr>
<tr>
<td>BC + virginiamicin</td>
<td>2.16b</td>
</tr>
<tr>
<td>SEM</td>
<td>0.08</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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.
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 promoter decrease intestine length. Visek (1978) indicated that, growth promoter 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 virginiamycin 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 different 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 + viriginiamycin. 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 parameters, digestibility and carcass development 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**


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


