Performance and Cecal Bacteria Counts of Broilers Fed Low Protein Diets With and Without a Combination of Probiotic and Prebiotic

ABSTRACT

A total of 360 one-day-old Cobb 500 chicks were randomly distributed in a completely randomized design according to a 3×2 factorial arrangement, consisting of three levels of dietary crude protein (100, 90 and 85% of NRC recommended levels) and a feed additive (with or without feed additive). A blend of a commercial probiotic and a prebiotic were used as feed additives. Each treatment had four replicates of 15 birds each. Prebiotic and probiotic were added to the starter (days 1 to 21) and finisher (days 22 to 42) diets according to the manufacturer’s recommendations. The findings indicated that significant differences were not observed between 100 and 90% NRC for broiler performance (body weight, body weight gain, feed intake and feed conversion ratio) throughout the experiment, while the birds fed the diets containing 85% NRC had poorer performance than those fed 100% (p<0.05). Feed additives had no significant effect on broilers performance. There was no significant interaction between protein level and feed additive for performance. Dietary inclusion of feed additives had no significant effect on cecal lactobacillus and Escherichia coli counts at 21 days of age, while, at 42 days of age, feed additive increased lactobacillus and decreased the counts of Escherichia coli (p<0.05). In conclusion, according to the findings of the current experiment, dietary crude protein could be reduced by 10%, without negative effect on broiler performance. Supplementation with feed additives had no significant effect on broiler performance, but beneficially influenced cecal bacteria counts at 42 days of age.

INTRODUCTION

The major cost in poultry nutrition is related to feed ingredients, particularly protein and energy sources (Wijtten et al., 2004). Broiler chickens require high level of dietary protein and their performance is severely influenced by this nutrient. Due to environmental concerns related to high nitrogen excretion, as well as high price of dietary protein sources, application of low protein diets (LPD) in poultry nutrition has received more attention in recent years (Amirdahri et al., 2012). Also, application of LPD will allow the producer to use alternate feedstuffs (Kamran et al., 2008). It is suggested that the level of dietary protein in starter, grower and finisher phases of the rearing period could be reduced by 10% from the NRC (1994) recommendation levels, without adverse effects on broilers performance, however, excessive reduction in dietary protein levels can cause lower performance and carcass yield (Aftab et al., 2006), as shown in some studies (Houshmand et al., 2012a, b).

On the other hand, in recent years, dietary inclusion of antibiotic growth promoters has been banned in some regions of the world.
Hence, different organic feed additives such as probiotics and prebiotics have been proposed as alternatives to antibiotics. Probiotic is defined as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance” (Fuller, 1989). Prebiotics are nondigestible carbohydrates such as fructooligosaccharides and mannanoligosaccharides that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gut (Gibson & Roberfroid, 1995).

Supplementation with probiotics and prebiotics can improve the performance of broiler chickens (Kim et al., 2011; Yang et al., 2012; Houshmand et al., 2012b; Salim et al., 2013; Bozkurt et al., 2014). Also, their positive effects on protein utilization have been indicated. Angel et al. (2005) found that broilers fed diets containing lower levels of nutrients (protein, calcium and available phosphorus) had poorer performance than the control group, but supplementation of those diets with probiotic, improved nutrients retention and thus prevented their negative effects. In another study, it was shown that supplementation with a prebiotic, improved the performance of broilers fed with LPD (Torres-Rodriguez et al., 2005). Considering the beneficial influences of probiotics and prebiotics on protein utilization and, the more efficiency of feed additives under suboptimal nutrition conditions (Torres-Rodriguez et al., 2005), it is expected that dietary inclusion of these additives reduce the negative effects of LPD.

Gut microflora play a very important role in the host’s nutrition and health. It is reported that intestinal microflora balance is beneficially influenced by probiotics and prebiotics. A mixture of probiotic and prebiotic is known as symbiotic. Probably, symbiotic has more positive effects on animals compared with probiotic or prebiotic alone (Yang et al., 2009). Different commercial probiotics and prebiotics are used by Iranian producers. However, information on the possibility of use of a mixture of these two additives as symbiotic is limited. Hence, the current study was designed to determine the effects of a mixture of a probiotic and a prebiotic on performance and cecal bacteria of broilers fed with different levels of protein.

**MATERIALS AND METHODS**

All procedures used in the current experiment were approved by the Institution of Animal Care Committee, of the Yasouj University. The study was conducted as a 3 × 2 factorial arrangement of three levels of dietary crude protein with and without feed additive. The three dietary protein levels were: 100, 90 and 85% of NRC recommended levels. A mixture of a commercial probiotic (Primalac, Star-Labs, USA, containing *Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium, Bifidobacterium bifidium*) and a prebiotic (Fermacto, the commercially available fermentation product of *Aspergillus orizae*, Pet-Ag, Ltd) at the ratio of 1:1 were included in experimental diets as feed additives.

A total of 340 one-day-old male and female Cobb 500 broiler chicks were purchased from a commercial local hatchery and transferred to a rearing place. At arrival time, they were randomly allocated to 1 of 6 experimental treatments with four replicates and 15 birds per replicate. Isocaloric starter and finisher diets were formulated to meet or exceed the NRC (1994) nutrients requirements (except for crude protein and essential amino acids) and were fed from 1 to 21 and 22 to 42 days of age, respectively. The composition of the experimental diets is shown in Table 1. Experimental diets and water were provided *ad libitum* the entire study. The birds were reared in floor pens (150 cm length × 150 cm width) with rice straw as litter under similar management condition. Feed intake was calculated weekly on a pen basis. Birds in each pen were weighed as a group at 21 and 42 days of age and FCR was calculated as the ratio of feed intake to body weight gain. Mortality was recorded daily and FCR was adjusted for mortality.

At the end of the starter and finisher phases of the study (days 21 and 42, respectively), one bird from each pen was sacrificed by cervical dislocation and immediately, the digestive system was carefully removed and the weights of the thighs, breast, liver and abdominal fat were measured. The relative organ weight was calculated and expressed as the weight of the organ as a percentage of live body weight. In addition, at the same time (days 21 and 42), cecal digesta samples were taken and transferred to the sterile tubes and placed on ice and immediately sent to the Microbiology Lab to determine the counts of *Escherichia coli* and *Lactic acid bacteria*. Each sample was serially diluted from initial 10⁻¹ to 10⁻⁸. Then, 100 µL of diluted samples were plated on the Eosin Methyleyne Blue (EMB) (for *E. Coli*) and De Man, Rogosa and Sharpe (MRS) (for *Lactobacillus*) agar media. Finally, EMB and MRS media were incubated at 37º C for 24 and 48 hours under anaerobic and aerobic conditions, respectively. The results are shown as colony forming unit (CFU) per gram of cecal digesta.
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Data was analyzed by analysis of variance using the General Linear Models (GLM) procedures of SAS software (SAS Institute, 2005). The means were compared by Duncan’s multiple range test. The level of statistical significance was set at p< 0.05.

RESULTS AND DISCUSSION

Performance

The effects of protein level and feed additives on broiler performance (Table 2) indicated that starter (days 1-21) body weight gain was not influenced significantly by protein level. Thus, there was no significant difference in body weight among the three levels of dietary protein at 21 days of age. However, finisher (days 22-42) body weight gain was higher in birds fed 100 and 90% NRC diets, compared with those fed 85% NRC diet. So, the birds of 85% NRC had lower final body weight compared to NRC and 90% NRC. Similarly, overall (days 1-42) body weight gain was lower in 85% NRC than the other two levels. During the starter phase, feeding with diets containing 85% NRC resulted in a lower feed intake than the 90% NRC. Also, birds in 85% NRC had lower overall feed intake than the NRC and 90% NRC. Protein levels had no significant effect on the starter FCR. However, during the finisher phase, birds of 85% NRC had worse FCR than the other two levels. This impaired FCR, resulted in the worst overall (days 1-42) FCR in the 85% NRC level.

The current results showed that significant differences in performance traits were not observed between NRC and 90% NRC. That means that dietary protein levels could by decreased by 10 percent without deleterious effects on broiler performance. As stated earlier, such diets have important advantages (less environmental pollution, lower cost and increased flexibility in feed formulation).

In a review study, Aftab et al. (2006) investigated the results of different studies on the effects of LPD on broilers. They concluded that the level of dietary crude protein in 3 phases of the rearing period (starter, grower and finisher) could be reduced by 10 percent without deleterious effects on broiler performance. Further reduction in dietary protein level will impair the performance traits as well as carcass characteristics.

Birds fed with diets containing 85% NRC had poorer performance than those fed with the recommended level of NRC. On the other hand, these findings

| Ingredients (%) | %100 NRC | 90% NRC | 85% NRC |
|-----------------|----------|---------|---------|
| Corn            | 60.19    | 63.81   | 65.61   |
| Soybean meal(44%CP) | 34.85   | 29.30   | 26.60   |
| Vegetable oil   | 1        | 1       | 1       |
| Limestone       | 1.10     | 1.10    | 1.20    |
| Dicalcium phosphate | 1.80    | 1.80    | 1.80    |
| Common salt     | 0.36     | 0.36    | 0.36    |
| Vitamins premix2 | 0.25    | 0.25    | 0.25    |
| Minerals premix3 | 0.25    | 0.25    | 0.25    |
| DL-Methionine   | 0.20     | 0.18    | 0.18    |
| L-Lysine        | -        | 0.05    | 0.05    |
| Inert material (Sand) | -      | 1.90    | 2.70    |

1diets containing three levels of protein: 100% NRC recommendation, 90% of NRC recommendation; 85% of NRC recommendation.

The vitamin premix supplied the following per kilogram of diet: vitamin A (retinyl acetate), 8,000 IU; vitamin D3, 1,000 IU; vitamin E (dl-α-tocopherol), 30 IU; vitamin K3, 2.5 mg; vitamin B1, 2 mg; vitamin B2, 5 mg; vitamin B6, 2 mg; vitamin B12, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg, folic acid, 0.5 mg.

The mineral premix supplied the following per kilogram of diet: Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg; Se, 0.25 mg; Co, 0.2 mg.

Chemical composition

| ME (Kcal/kg) | 2887 | 2887 | 2887 | 3000 | 3000 | 3000 |
|--------------|------|------|------|------|------|------|
| Crude protein (%) | 20.74 | 18.66 | 17.63 | 18.75 | 16.88 | 15.94 |
| Calcium (%) | 0.91 | 0.91 | 0.91 | 0.85 | 0.85 | 0.85 |
| Available phosphorus (%) | 0.49 | 0.49 | 0.49 | 0.33 | 0.33 | 0.33 |
| Lysine (%) | 0.99 | 0.89 | 0.84 | 0.94 | 0.85 | 0.80 |
| Methionine (%) | 0.45 | 0.41 | 0.38 | 0.36 | 0.32 | 0.31 |
| Arginine (%) | 1.32 | 1.17 | 1.08 | 1.03 | 0.93 | 0.88 |
| Threonine (%) | 0.72 | 0.65 | 0.61 | 0.69 | 0.62 | 0.58 |
| Tryptophan (%) | 0.29 | 0.26 | 0.24 | 0.17 | 0.15 | 0.14 |
indicate that reduction in dietary protein level by 15% is severe and will impair broilers performance. Hence, it is not recommended. Considering the important role of adequate levels of dietary protein and amino acids on broilers performance (NRC 1994; Kamran et al., 2008), these results are not surprising. In line with our results, deleterious effects of LPD on broilers performance have been shown in some previous studies (Bregendahl et al., 2002; Kamran et al., 2008; Houshmand et al., 2012a, b).

If dietary crude protein level is lowered by more than three percent, broilers performance and also carcass composition are adversely influenced (Bregendahl et al., 2002; Waldroup et al., 2005). The effect of LPD with constant Metabolizable Energy: Crude Protein (ME:CP) ratio on performance and carcass characteristics of broilers were studied by Kamran et al. (2008). Their results indicated that feeding with low protein and low energy diets reduced the broilers performance (feed intake, body weight gain and feed conversion ratio) as well as protein and energy efficiency ratio during grower, finisher and overall experimental periods. Lower efficiency of dietary energy and protein utilization and also inadequate levels of one or more less-essential amino acids like Arg, Ile and Val in the LPD, were suggested as possible reasons for poorer performance of birds fed on low diets. In three experiments conducted by Bregendahl et al. (2002), broilers fed LPD had less body weight gain, worse FCR and inferior N retention than those fed with the control diets. Waldroup et al. (2005) found that broilers fed with LPD (16, 18 and 20% CP) had inferior performance (decreased body weight gain and increased feed conversion ratio) than those fed with the control diets (24% CP) in the starter phase.

Reasons for the reduction in performance at LPD have not been totally explained (Waldroup et al., 2005) and more studies are needed to determine the responsible reason (or reasons) in this case (Bregendahl et al. 2002). Possible reasons are as follows: change in dietary potassium or the dietary electrolyte balance, insufficiency of the non-specific nitrogen for the synthesis of non essential amino acids, tendency of broilers to reduce the voluntary feed intake on low protein diets, altered essential amino acids/non essential amino acids ratio, insufficient synthesis of non essential amino acids like glycine to fulfill the need of fast growing broilers, efficiency of utilization of amino acids from a free source vs. intact dietary protein for body protein accretion, insufficiency of some of the essential amino acids, relationship between the dietary metabolizable energy and the net energy of LPD vs. control/high-protein diets (Aftab et al., 2006).

The results of the current study (Table 2) showed that broilers performance were not influenced by feed additives throughout the study. There are conflicting results on the efficacy of probiotic, prebiotic and symbiotic on performance of broilers (Yang et al., 2009). In a recent study, the effects of a commercial probiotic, prebiotic and their combination (symbiotic)
on broilers performance during the first two weeks of age were investigated by Murshed & Abudabos (2015). They found that dietary inclusion of probiotic and prebiotic had beneficial effects on body weight gain and FCR, while such positive effects were not observed for symbiotic. In contrast, in the study of Murarolli et al. (2014), symbiotic had more growth promoting effects (more body weight gain and better FCR) compared with probiotic and prebiotic. In another study, broilers were reared on the reused litter from a commercial broiler flock (as a natural health challenge) and received diets supplemented with probiotic, prebiotic, symbiotic and organic acid. Dietary inclusion of probiotic, prebiotic and symbiotic resulted in a better performance during the first days of rearing period (days 1-10). However, overall (days 1-42) performance was not influenced by these additives (Fernandes et al., 2014). Awad et al. (2009) found that birds fed with diets supplemented with symbiotic had higher body weight and better FCR compared to those fed with the control and probiotic diets. Probiotic also had a growth-promoting effect, but lower than symbiotic.

In the current study, significant interaction between protein level and feed additives was not observed for performance traits. This means that broiler's response to feed additives was not influenced by dietary protein level. In agreement with this result, Navidshad et al. (2010) did not find significant interaction between prebiotic (Fermacto) and dietary nutrients level for performance traits, when broilers were given diluted diets from 10 to 21 days of age. In another study, the effects of 3 levels of crude protein (high: 24%, from 0 to 42 days of age; low: 22.08% from 0 to 42 days of age and medium: 24% from 0 to 21 days and 22.08% from 22 to 42 days of age) and three levels of symbiotic (without, recommended and 150% of recommended levels) on performance of Japanese quails were studied by Sharifi et al. (2011). Poorer performance was observed in birds fed LPD compared with other diets. Symbiotic had no significant effect on feed conversion ratio, feed intake and body weight. Also, there was no significant interaction between protein level and symbiotic for performance traits. They concluded that under conditions of good hygiene, dietary supplementation with symbiotic had no beneficial consequences on quail performance. In contrast to our results, Torres-Rodriguez et al. (2005) reported that dietary inclusion of a prebiotic (Fermacto) did not improve the weight gain of broilers fed with normal levels of dietary protein from 1 to 21 days of age, while it had beneficial effects on body weight gain of birds fed with LPD. Angel et al. (2005) reported that feeding with diets containing medium and low levels of dietary nutrients (protein, calcium and available phosphorus) impaired broilers performance. However, dietary addition of a Lactobacillus-based probiotic prevented the negative effects of diets containing medium levels of nutrients.

Bird's characteristics (age, species, and production stage), nutrition, environment, management, type of additives and its dosage (Yang, et al., 2009), environmental stressors (high relative humidity and also high environmental temperature), rearing system (cage or floor pen) and the number of experimental birds (Houshmand et al., 2012a) are important factors influencing broilers responses to prebiotics. When birds are reared under suboptimal experimental conditions, feed additives are more efficient (Orban et al., 1997). Growth-promoting effects of probiotic and prebiotic were more pronounced under coccidial challenge conditions rather than under unchallenged conditions (Bozkurt et al., 2014). In addition, it is reported that under clean or hygienic condition, broilers do not need any feed additives for maximum growth (Baurhoo et al., 2009). The non significant effects of additives such as prebiotic or antibiotic on broilers performance could be attributed to the lack of a real microbial challenge in rearing condition (Morales-Lopez et al., 2009).

Effectiveness of probiotics is influenced by many factors such as nutrition, environment (hygienic condition) and management. Rearing condition, probiotic dosage, bird's age as well as the delivery method (water or feed) can also affect broilers performance and gut bacterial responses to this feed additive (Yang et al., 2009). Hence, it is difficult to compare the results of different studies (Yang et al., 2012). All above-mentioned factors probably can explain the contrasting results in the performance of broilers fed with probiotic and prebiotic. Therefore, the differences in our results and others could be attributed to them.

Cecal bacteria counts

The effects of protein level and feed additive on cecal bacteria counts are presented in Table 3. At 21 days of age, Lactobacillus and Escherichia coli counts were significantly not influenced by protein level or feed additives. However, at the end of the experiment (day 42), the feed additives had beneficial effects on cecal bacteria population. Supplementation with feed additives increased significantly the number of lactobacillus but decreased the Escherichia coli.
counts. Salim et al. (2013) reported that dietary supplementation with a probiotic did not influence the cecal *Lactobacillus* and *Salmonella* content. However, the number of cecal *Escherichia coli* decreased significantly in birds fed with probiotic. In another study (Yang et al., 2012), dietary addition of a probiotic (*Clostridium butyricum*) decreased the counts of *Escherichia coli, Salmonella* and *Clostridium perfringens* in cecal contents of broilers. Also, cecal *Lactobacillus, Bifidobacterium* and *C. butyricum* counts were higher in birds fed with supplemented diets, compared with those in the control group. Kim et al. (2011) found that dietary supplementation with different prebiotics (0.25% FOS and 0.05% MOS) increased *Lactobacilli* count and decreased the number of *Clostridium perfringens* and *Escherichia coli* in small intestine of broilers reared up to 28 days of age.

Different mechanisms have been suggested for the effects of probiotics on the gut microflora: competitive exclusion, lowering the pH through acid fermentation, competition for mucosal attachment sites and nutrients, production of bacteriocins, stimulating the immune system associated with the gut, increasing production of short-chain fatty acids, increasing epithelial integrity, reducing epithelial cell apoptosis and stimulating the intra-epithelial lymphocytes (Salim et al., 2013). Dietary addition of probiotic can result in higher levels of short-chain fatty acids (Acetic, butyric, valeric and total fatty acids) in broilers cecum. As a result, pH of cecal digesta will decrease. This condition is favorable for growth of beneficial bacteria, but it is unfavorable for pathogenic bacteria (Yang et al., 2012).

**Thigh, breast, liver and abdominal fat weights**

The effects of protein level and feed additives on breast, femur, liver and abdominal fat weights (Table 4) indicated that these parameters were not influenced significantly by protein level or feed additives. It is stated that LPD contains higher ME:CP ratio than the normal

### Table 3 – Effects of feed additive and protein level on cecal bacterial counts [log (cfu/g)] at 21 and 42 d of age

| Parameter            | Protein level¹ | Additive² | SEM | SEM | Interaction |
|----------------------|----------------|-----------|-----|-----|-------------|
|                      | 100% NRC      | 90% NRC   | 85% NRC |     |
| *Lactobacillus*      |               |           |      |     |
| d 21                 | 9.8           | 10.5      | 10.3 | 0.46| 10.5        |
| d 42                 | 11.6          | 11.7      | 11.7 | 0.57| 12.0b       |
| *Escherichia coli*   |               |           |      |     |
| d 21                 | 8.2           | 9.0       | 9.3  | 0.66| 8.9         |
| d 42                 | 11            | 10.6      | 11.1 | 0.44| 9.9a        |

¹three dietary protein levels: 100% NRC recommendation; 90% of NRC recommendation; 85% of NRC recommendation.

²with feed additive: +; without feed additive: –

NS: not significant

SEM: standard error of the mean

Means followed by the same letter in the row do not differ by Duncan test (p>0.05)

### Table 4 – Effects of feed additive and protein level on relative weights (% body weight) of thigh, breast, liver and abdominal fat weight at 21 and 42 d of age

| Parameter            | Protein level¹ | Additive² | SEM | SEM | Interaction |
|----------------------|----------------|-----------|-----|-----|-------------|
|                      | 100% NRC      | 90% NRC   | 85% NRC |     |
| Thigh                |               |           |      |     |
| d 42                 | 18.4          | 18.3      | 17.6 | 3.8 | 17.9        |
| d 42                 | 21.7          | 19.3      | 18.2 | 2.1 | 19.6        |
| Breast               |               |           |      |     |
| d 42                 | 2.88          | 3.17      | 3.52 | 0.30| 3.19        |
| d 42                 | 2.60          | 2.85      | 2.91 | 0.17| 2.89        |
| Liver                |               |           |      |     |
| d 21                 | 1.43          | 1.54      | 1.57 | 0.23| 1.49        |
| d 42                 | 2.26          | 1.89      | 2.20 | 0.51| 2.34        |
| Abdominal fat pad    |               |           |      |     |

¹three dietary protein levels: 100% NRC recommendation; 90% of NRC recommendation; 85% of NRC recommendation.

²with feed additive: +; without feed additive: –

NS: not significant

SEM: standard error of the mean

Means followed by the same letter in the row do not differ by Duncan test (p>0.05)
diets. Feeding with such diets will increase lipogenesis in the liver of the birds, thereby causing in more liver weight and hence more abdominal fat deposition (Kamran et al., 2008). However, such effect was not observed in the current study. In line with our results, a previous study indicated that feeding with LPD had no significant effect on breast meat, thigh, abdominal fat and liver weights. It was possible that LPD contained adequate levels of essential amino acids, particularly lysine and methionine. As these two amino acids are exclusively used for protein accretion in the body, the non significant difference between birds fed with low and normal protein diets was attributed to this factor (Kamran et al., 2008). In addition, Sharifi et al. (2011) reported that breasts and thighs of Japanese quails were not influenced by dietary crude protein levels or symbiotic.

CONCLUSIONS

Dietary crude protein could be reduced by 10% from the NRC recommended levels, without negative effect on broilers performance. Performance was significantly not influenced by feed additives, but the additives had beneficial consequences on cecal bacteria counts at the end of the experiment. Also, broiler response to feed additives was not influenced by dietary protein level.

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