Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Ravangard, AH; Houshmand, M; Khajavi, M; Naghiha, R

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

Revista Brasileira de Ciência Avícola, vol. 19, enero-marzo, 2017, pp. 75-82
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Jan - Mar 2017

Special Issue Nutrition / 075-082

http://dx.doi.org/10.1590/1806-9061-2016-0319

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

Author(s)

Ravangard AH[I]
Houshmand M[I]
Khajavi M[I]
Naghiha R[I]

[I] Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran

Mail Address

Corresponding author e-mail address
Mohammad Houshmand
Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran
Postal code: 75918-74831
Tel: +987433224840
Email: hooshmand@yu.ac.ir

Keywords

Broiler, low protein diet, prebiotic, probiotic.

Submitted: June/2016
Approved: November/2016

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 NRCrecommended 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 prbiotics 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 libitm* 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^{-9}. Then, 100 µL of diluted samples were plated on the Eosin Methelyne Blue (EMB) (for *E. Coli*) and De Man, Rogosa and Sharpe (MRS) (for *Lactobacillus*) agar media. Finally, EMB and MRS media were incubated at 37° 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.

Table 1 – Composition of the experimental diets

Ingredients (%)	Starter[1]			Finisher[1]		
	%100 NRC	90% NRC	85% NRC	%100 NRC	90% NRC	85% NRC
Corn	60.19	63.81	65.61	65.67	69.29	70.85
Soybean meal(44%CP)	34.85	29.30	26.60	29.85	24.87	22.45
Vegetable oil	1	1	1	1.13	1	1
Limestone	1.10	1.10	1.20	1.33	1.34	1.34
Dicalcium phosphate	1.80	1.80	1.80	1.14	1.20	1.24
Common salt	0.36	0.36	0.36	0.32	0.32	0.32
Vitamins premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
Minerals premix[3]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.18	0.18	0.06	0.05	0.04
L-Lysine	-	0.05	0.05	-	-	0.02
Inert material (Sand)	-	1.90	2.70	-	1.43	2.24
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

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

[2]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.

[3]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.

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% from the respective NRC (1994) recommendation levels, without deleterious consequences on broilers 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

Table 2 – Effects of feed additive and protein level on body weight, body weight gain, feed intake and FCR of broilers

Parameter	Protein level[1]			SEM	Additive[2]		SEM	Interaction
	100% NRC	90% NRC	85% NRC		+	-		
Body weight (g)								
d 21	633	704	568	43	643	639	35	NS
d 42	1963[a]	1891[a]	1360[b]	85	1657	1744	69	NS
Body weight gain (g)								
d 1-21	589	661	525	43	599	595	35	NS
d 22-42	1330[a]	1187[a]	792[b]	99	1014	1105	81	NS
d 1-42	1916[a]	1848[a]	1317[b]	85	1613	1700	69	NS
Feed intake (g)								
d 1-21	1042[ab]	1106[a]	975[b]	33	1038	1031	27	NS
d 22-42	3055	3056	2645	186	2754	3031	152	NS
d 1-42	4097[a]	4162[a]	3620[b]	169	3792	4062	144	NS
Feed conversion ratio								
d 1-21	1.77	1.67	1.84	0.09	1.73	1.73	0.07	NS
d 22-42	2.30[b]	2.57[b]	3.34[a]	0.14	2.72	2.74	0.11	NS
d 1-42	2.14[b]	2.25[b]	2.75[a]	0.08	2.35	2.39	0.06	NS

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

[2] with 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 ($p>0.05$)

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 a*l. (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, prebiotc 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 conversation 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*

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

Parameter	Protein level[1]			SEM	Additive[2]		SEM	Interaction
	100%NRC	90% NRC	85% NRC		+	-		
Lactobacillus								
d 21	9.8	10.5	10.3	0.46	10.5	10.1	0.46	NS
d 42	11.6	11.7	11.7	0.57	12.0[a]	11.4[b]	0.56	NS
Escherichia coli								
d 21	8.8	9.0	9.3	0.66	8.9	9.2	0.67	NS
d 42	11	10.6	11.1	0.44	9.9[b]	11.5[a]	0.14	NS

[1]three dietary protein levels: 100% NRC recommendation; 90% of NRC recommendation; 85% of NRC recommendation.
[2]with 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)

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 perfringen* 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 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 level[1]			SEM	Additive[2]		SEM	Interaction
	100% NRC	90% NRC	85% NRC		+	-		
Thigh								
d 42	18.4	18.3	17.6	3.8	17.9	18.4	3.8	NS
Breast								
d 42	21.7	19.3	18.2	2.1	19.6	19.9	2.1	NS
Liver								
d 21	2.88	3.17	3.52	0.30	3.19	3.20	0.30	NS
d 42	2.60	2.85	2.91	0.17	2.89	2.68	0.17	NS
Abdominal fat pad								
d 21	1.43	1.54	1.57	0.23	1.49	1.50	0.23	NS
d 42	2.26	1.89	2.20	0.51	2.34	1.90	0.51	NS

[1]three dietary protein levels: 100% NRC recommendation; 90% of NRC recommendation; 85% of NRC recommendation.
[2]with 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.

ACKNOWLEDGMENTS

The authors would like to thank the Yasouj University, Iran for the providing supports for this study.

REFERENCES

Aftab U, Ashraf M, Jiang Z. Low protein diets for broilers. World's Poultry Science Journal 2006;62(4):688-701.

Amirdahri S, Janmohammadi H, Taghizadeh A, Rafat A. Effect of dietary *Aspergillus* meal prebiotic on growth performance, carcass characteristics, nutrient digestibility, and serum lipid profile in broiler chick low-protein diets. Turkish Journal of Veterinary and Animal Science 2012;36(6):602-610.

Angel R, Dalloul RA, Doerr J. Performance of broiler chickens fed diets supplemented with adirect-fed microbial. Poultry Science 2005;84(8):1222–1231.

Awad WA, Ghareeb K, Abdel-Raheem S, Böhm J. Effects of dietary inclusion of probiotic and synbiotic on growth performance, organ weights, and intestinal histomorphology of broiler chickens. Poultry Science 2009;88(1):49–55.

Baurhoo B, Goldflus F, Zhao X. Purified cell wall of *Saccharomycescerevisiae* increases protection against intestinal pathogens in broiler chickens. International Journal of Poultry Science 2009;8(2):133–137.

Bozkurt M, Aysul N, KüçüKyilmaz K, Aypak S, Ege G, Çatli AU, et al. Efficacy of in-feed preparations of an anticoccidia, multienzyme, prebiotic, probitoc, and herbal essential oil mixture in healthy and *Eimeria* spp.-infected broilers. Poultry Science 2014;93(2):389-399.

Bregendahl K, Sell JL, Zimmerman DR. Effect of low-protein diets on growth performance and body composition of broiler chicks. Poultry Science 2002;81(8):1156-1167.

Fernandes BCS, Martins MRFB, Mendes AA, Milbradt EL, Sanfelice C, Martins BB, et al. Intestinal integrity and performance of broiler chickens fed a probiotic, a prebiotic, or an organic acid. Brazilian Journal of Poultry Science 2014;16(4):417-424.

Fuller R. Probiotics in man and animals. Journal of Applied Bacteriology1989;66(5):365-378.

Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. Journal of Nutrition 1995;125(6):1401–1412.

Houshmand M, Azhar K, Zulkifli I, Bejo MH, Kamyab A. Effects of prebiotic, protein level, and stocking density on performance, immunity and stress indicators of broilers. Poultry Science 2012a;91(2):393-401.

Houshmand M, Azhar K, Zulkifli I, Bejo MH, Kamyab A. Effects of non-antibiotic feed additives on performance, immunity and intestinal morphology of broilers fed different levels of protein. South African Journal of Animal Science 2012b;42(1):22-32.

Kamran Z, Sarwar M, Nisa M, Nadeem MA, Mahmood S, Babar ME, et al. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. Poultry Science 2008;87(3):468-474.

Kim GB, Seo YM, Kim CH, Paik IK. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poultry Science 2011;90(1):75-82.

Morales-Lopez R, Auclair E, Garcia F, Esteve-Garcia E, Brufau J. Use of yeast cell walls;β-1, 3/1, 6-glucans;and mannoproteins in broiler chicken diets. Poultry Science 2009;88(3):601–607.

Murarolli VDA, Burbarelli MFC, Polycarpo GV, Ribeiro PAP, Moro MEG, Albuquerque R. Prebiotic, probiotic and symbiotic as alternative to antibiotics on the performance and immune response of broiler chickens. Brazilian Journal of Poultry Science 2014;16(3):279-284.

Murshed MA, Abudabos AM. Effects of the dietary inclusion of a probiotic, a prebiotic or their combinations on the growth performance of broiler chickens. Brazilian Journal of Poultry Science 2015;17(special issue):99-103.

Navidshad B, Adibmoradi M, Ansari Pirsaraei Z. Effects of dietary supplementation of Aspergillus originated prebiotic (Fermacto) on performance and small intestinal morphology of broiler chickens fed diluted diets. Italian Journal of Animal Science 2010;9(1):55-60.

NRC. Nutrient requirements of poultry. Washington: National Academy Press; 1994.

Orban J, Patterson J, Sutton A, Richards G. Effect of sucrose thermal oligosaccharide caramel, dietary vitamin-mineral level, and brooding temperature on growth and intestinal bacterial populations of broiler chickens. Poultry Science 1997;76(3):482–490.

Salim HM, Kang HK, Akter N, Kim DW, Kim JH, Kim MJ, et al. Supplementation of direct fed microbials as an alternative to antibiotic on growth performance, immune responses, cecal microbial population, and ileal morphology of broiler chickens. Poultry Science 2013;92(8):2084–2090.

SAS. User's guide. Version 9. Cary: SAS Institute; 2005.

Sharifi MR, Shams Shargh M, Dastar B, Hassani S. The effect of dietary protein levels and synbiotic on performance parameters, blood characteristics and carcass yields of Japanese quail (*Coturnix coturnix Japonica*). Italian Journal of Animal Science 2011;10(1):17-21.

Torres-Rodriguez A, Sartor C, Higgins SE, Wolfenden AD, Bielke LR, Pixley CM, et al. Effect of aspergillus meal prebiotic (Fermacto) on performance of broiler chickens in the starter phase and fed low protein diets. Journal of Applied Poultry Research 2005;14(4):665–669.

Yang CM, Cao GT, Ferket PR, Liu TT, Zhou L, Zhang L, et al. Effects of probiotic, Clostridium butyricum, on growth performance, immune function, and cecal microflora in broiler chickens. Poultry Science 2012;91(9):2121–2129.

Yang Y, Iji PA, Choct M. Dietary modulation of gut microflora in broiler chickens: a review of the role of six kinds of alternatives to in-feed antibiotics. World's Poultry Science Journal 2009;65(1):97-114.

Waldroup PW, Jiang Q, Fritts CA. Effects of supplementing broiler diets low in crude protein with essential and nonessential amino acids. International Journal of Poultry Science 2005;4(6):425-431.

Wijtten PJA, Lemme A, Langhout DJ. Effects of different dietary ideal protein levels on male and female broiler performance during different phases of life:Single phase effects, carryover effects and interactions between phases. Poultry Science 2004;83(12):2005-2015.