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The Effects of Phytase and Beta-Glucanase Supplementation on Performance, Egg Quality, Some Blood Parameters, Tibia and Excreta Characteristics of Japanese Quails Fed Barley-Based Diets§

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Abstract

This study was conducted to investigate the effects of phytase and β-glucanase addition to barley-based diets which include different protein levels on performance, egg weight, egg mass, some egg quality parameters, serum total protein, calcium and phosphorus levels, some tibia and excreta characteristics of quails.In this research, a total of 360, 10 weeks old Japanase quails (*Coturnix coturnix japonica*) were randomly distributed into 3x2x2 completely randomized factorial arrangement with 12 groups having 10 replicates in each content 3 Japanase quails. Quails were fed phytase (0.06 % of diet) and β-glucanase (0.005 % of diet) and their combination in three levels of crude protein (16, 18 and 20%) and 2850 kcal kg^{-1} metabolisable energy from 10 to 20 weeks of age.At the end of the study, there were no differences between body weight, feed intakes, feed conversion ratios, egg production and quality, serum calcium and phosphorus levels, all of tibia characteristics and excreta ash percentages. Egg weight and serum total protein level were increased by the crude protein level of experimental diets increased as well (P<0.01). β-glucanase supplementation improved egg mass (P<0.01) and increased calcium excretion (P<0.05). In this study, phytase addition had a positive effect on the environment since phosphorus excretion was approximately decreased 16% (P<0.01). In conclusion, crude protein level in barley-based layer diets should not be lower than 18% for optimum egg weight of Japanese quails and enzyme supplementation provided some positive effects on some criteria evaluated in this study.

Key words: Enzyme, protein, serum, quail, tibia

Arpa Ağırlıklı Rasyonlara Fitaz ve Betaglukanaz İlavesinin Japon Bıldırcınlarında Performans, Yumurta Kalitesi, Bazı Kan Parametreleri, Tibia ve Dışkı Kriterleri Üzerine Etkileri

Özet

Bu çalışma, farklı protein içerikli arpa ağırlıklı bıldırcın rasyonlarına fitaz ve β-glukanaz ilavesinin performans, yumurta ağırlığı, kütlesi ve bazı kalite özellikleri, serum total protein, kalsiyum (Ca), fosfor (P) seviyesi, bazı tibia ve dışkı kriterleri üzerine etkilerini belirlemek amacıyla yapılmıştır. Araştırmada 10 haftalık yaşta toplam 360 adet Japon bıldırcını (*Coturnix coturnix japonica*), 3x2x2 faktöriyel deneme deseninde 12 farklı grupta 10 tekerrürlü ve her tekerrürde 3 hayvan olacak şekilde rastgele dağıtılmışlardır. Bıldırcınlar 10 - 20 haftalık yaşlar arasında % 16, 18 ve 20 ham protein (HP) ve 2850 kcal kg^{-1} metabolik enerjili ayrıca her bir HP seviyesinde fitaz (% 0.06), β-glukanaz (% 0.005) ve fitaz + β-glukanaz ilaveli yemlerle beslenmişlerdir.Araştırma sonunda gruplar arasında canlı ağırlık, yem tüketimi, yem dönüşüm oranı, yumurta verimi ve kalitesi, serum Ca ve P seviyesi, tüm tibia kriterleri ve dışkı kül oranı bakımından istatistik önemli farklılık bulunmamıştır. Artan HP seviyesi, yumurta ağırlığı ve serum total protein seviyesini artırmıştır (P<0.01). β-glukanaz ilavesi, yumurta kütlesi (P<0.01) ile dışkıyla Ca atılımını artırmıştır (P<0.05). Fitaz ilavesi ise dışkı ile P atılımını yaklaşık % 16 oranında azaltarak çevre üzerine olumlu bir etki yapmıştır (P<0.01).Sonuç olarak, yumurta döneminde arpa ağırlıklı bıldırcın rasyonlarında optimum yumurta ağırlığı için rasyon HP içeriğinin % 18'in altına düşürülmemesi gerektiği, bu koşullar altında enzim ilavesinin bazı kriterler üzerinde olumlu katkılar sağladığı belirlenmiştir.

Anahtar kelimeler: Bıldırcın, enzim, protein, serum, tibia

§This article was summarized by first author's Phd thesis.

Introduction

Barley and wheat are used as alternative sources of energy for the feed industry in case of an increase in prices of maize and thus animal product cost. The cereals such as barley, wheat, rye and oat used in poultry rations have non-starch polysaccharides (NSP; β-glucan in barley and oat, arabinoxylan in wheat etc.) in their structure. Because poultry do not have the enzymes to break down the β-glucan, arabinoxylan and cellulose, they cannot get benefit from feeds such as barley and wheat. NSP adversely affects the performance of poultry. The addition of the appropriate enzymes into the feed could eliminate the negative effect of the NSPs. In order to break down the NSPs (which can be broken up in the small intestine with microbial fermentation and are soluble in the water) β-glucanase (which breaks down the β-glucans in barley and oat) and xylanase (which breaks down the arabinoxylan in wheat and rye) are added in poultry diets based on barley and wheat respectively (Perry, 1995; Choct, 2001; Leeson and Summers, 2001). Thanks to revealing the chemical structure of these compounds and enhanced enzyme technology, it has been possible to eradicate the adverse effects of NSPs (Williams et al., 1997). Hence, for the poultry producer, there is the opportunity for a more efficient feed conversion, heavier birds, better litter conditions, improved environmental control, animals' health and hygiene in the flock.

Enzymes also have positive effects on the availability of some minerals. Phosphorus (P) is one of the most important macro minerals for the poultry. However, most of the P in plant materials is found in a bound known as phytate (Punna and Roland, 1999) and due to the lack of activity of the endogenous phytase in the digestive system of birds, the availability of P is fairly low in poultry. For meeting the need of P, mostly inorganic P sources are used in poultry rations. However, a great deal of inorganic P is removed by excretion, which creates environmental pollution. Moreover, P source added to ration is the most expensive source after energy and protein, which directly increases the cost of animal production (Boling, 1999). In order to decrease these negative effects, the usage of phytase in poultry rations has become a widespread practice in recent years.

The negative effects of the modern poultry production systems on the environment have begun to attract attention particularly in recent years and there has been an increase in researches focusing on decreasing the quantity of nutrient excretion. One of the nutrients that negatively affect environment is nitrogen (N). It is reported that by reducing the ration CP content the amount of N excreted in environment has been diminished (Szczurek and Pisulewski, 1996; Kamran et al., 2010).

This research was conducted to identify the effects of two different enzymes, which use individual or combination with different protein levels on enzymes interaction, and their effects in animal metabolism of layer quails.

Materials and Methods

Animals and diets

In the research, a total of 360 Japanese quails (*Coturnix coturnix japonica*) at the age of 10 weeks were used. The quails were kept in layered system egg cages of 35 x 40 x 40 cm sizes, each pen containing 3 chicks, to receive 12 dietary treatments of barley soybean-based diets with 10 replicates of each treatment and at a temperature of 20 – 22 °C. During the research, 24 hour lightening was provided. The trial lasted 10 weeks. Feedstocks and experimental diets were analyzed in accordance with the methods defined in AOAC (2000), and the metabolic energy (ME) values were calculated from TSI (1991). The isocaloric (2850 kcal kg^{-1} ME) rations were arranged into three different crude protein (CP) levels (16, 18, and 20 %) and each diets at these protein levels included, no enzyme, β-glucanase (0.005 % of diet), phytase (0.06 % of diet) and (β-glucanase + phytase) supplementations, respectively. While the rations include an adequate level of Ca (2.5 %) as reported in the NRC (1994), the P was only provided by the feedstocks. The ingredients and chemical compositions of the rations used in the research were given in Tables 1 and 2. Pure β-glucanase was derived from *Trichoderma viride* (CBS 517.94) (8000 U g^{-1}) and provided by the Alltech Company with the commercial name Allzyme BG Concentrate; Phytase was originated from *Peniophora lycii* (500 FTU kg^{-1}) and was provided by the Trouw Nutrition TR Company with the commercial name Rovaphos. The manufacturers determined that the activities of the each enzymes. The experimental diets and water were offered ad-libitum.

The performance criteria

The total feed consumption was measured on a cage basis for each week. The egg production records were recorded daily in the experimental groups. Feed conversion ratio was calculated as grams of feed consumed per gram of egg produced.

Table 1. Ingredients of experimental diets (%)

Ingredient	C16	F16	B16	FB16	C18	F18	B18	FB18	C20	F20	B20	FB20
Barley	49.00	49.46	49.70	49.40	45.10	45.05	45.015	45.00	41.00	41.00	41.00	41.00
Maize	19.90	19.40	19.185	19.40	18.00	18.00	18.06	18.065	17.67	17.61	17.665	17.605
Soybean meal	17.54	17.50	17.50	17.525	23.30	23.30	23.30	23.30	26.60	26.60	26.60	26.60
Fish meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.40	2.40	2.40	2.40
Sunflower oil	5.05	5.07	5.10	5.10	5.38	5.37	5.40	5.35	5.40	5.40	5.40	5.40
Limestone	6.29	6.29	6.29	6.29	6.27	6.27	6.27	6.27	6.11	6.11	6.11	6.11
DL_Methionine	0.19	0.19	0.19	0.19	0.16	0.16	0.16	0.16	0.12	0.12	0.12	0.12
L-Lysine	0.19	0.19	0.19	0.19	0.03	0.03	0.03	0.03	-	-	-	-
L-Treonine	0.14	0.14	0.14	0.14	0.06	0.06	0.06	0.06	-	-	-	-
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vit. premiks[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Min. premiks[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Phytase	-	0.06	-	0.06	-	0.06	-	0.06	-	0.06	-	0.06
β-glucanase	-	-	0.005	0.005	-	-	0.005	0.005	-	-	0.005	0.005
TOTAL	100	100	100	100	100	100	100	100	100	100	100	100

C16: % 16 CP, without enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, without enzyme ; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, without enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.

[1]2.5 kg of vitamin premix provided: vit. A 12.000.000 IU, vit.D_3 3.000.000 IU, vit.E 30.000 mg, vit.K_3 3000 mg, vit.B_1 2500 mg, vit.B_2 6000 mg, niacin 35.000 mg, Ca-D-pantothenate 12.000 mg, vit B_6 4000 mg, vit B_{12} 15 mg, folic acid 1000 mg, biotine 45 mg, choline klorid 125.000 mg.

[2]1 kg of mineral premix provided; Fe 60.000 mg, Cu 5.000 mg, Mn 80.000 mg, , Co 200 mg, Zn 60.000 mg, I 1000 mg, Se 150 mg.

Table 2. Chemical composition of experimental diets

Nutrients	C16	F16	B16	FB16	C18	F18	B18	FB18	C20	F20	B20	FB20
ME[1], kcal/kg	2849	2845	2848	2847	2849	2846	2850	2845	2848	2845	2848	2846
Crude protein[1], %	16.09	16.02	16.03	16.07	18.10	18.09	18.06	18.05	20.00	20.10	20.04	20.02
Crude fat[1], %	7.20	7.20	7.20	7.20	7.50	7.50	7.50	7.40	7.50	7.50	7.50	7.50
Crude fiber[1], %	2.90	2.90	2.90	2.90	3.00	3.00	3.00	3.00	3.10	3.10	3.10	3.10
Crude ash[1], %	9.00	9.00	9.00	9.00	9.20	9.20	9.20	9.20	9.40	9.40	9.40	9.40
Calcium[1], %	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
T. Phosphorus[1], %	0.38	0.38	0.38	0.38	0.40	0.40	0.40	0.40	0.46	0.46	0.46	0.46
Av. Phosphorus[2], %	0.19	0.19	0.19	0.19	0.20	0.20	0.20	0.20	0.24	0.24	0.24	0.24
Methionine[2], %	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Met+sistin[2], %	0.73	0.73	0.73	0.73	0.76	0.76	0.76	0.76	0.78	0.78	0.78	0.78
Lysine[2], %	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.12	1.12	1.12	1.12
Arginine[2], %	1.00	1.00	1.00	1.00	1.15	1.15	1.15	1.15	1.30	1.30	1.30	1.30
Treonine[2], %	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.74	0.77	0.77	0.77	0.77
Triptofan[2], %	0.22	0.22	0.22	0.22	0.25	0.25	0.25	0.25	0.28	0.28	0.28	0.28
Linoleic acid[2], %	4.20	4.20	4.20	4.20	4.40	4.40	4.40	4.40	4.40	4.40	4.40	4.40

C16: % 16 CP, without enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, without enzyme ; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, without enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.

[1]Calculated with analysis value of feed ingredients.

[2]Values obtained from NRC (1994).

Egg weight, egg mass and some egg quality characteristics

To determine of egg weights, the total egg of groups in 15 days were kept in room temperature for 24 hours and their weights were taken by the precision balance. The egg masses were determined as g/ quail/ day by multiplying the average egg weight of experimental groups with the average egg production.

In the research, in order to find the egg quality parameters, 50 % of the eggs were chosen randomly among the eggs collected in two consecutive days and kept under room temperature for 24 hours, and then their shape index, shell thickness, shell rate and Haugh unit were defined.

$$\textit{Shape index (\%)} = \frac{\textit{Width of the egg (mm)}}{\textit{Length of the egg (mm)}} \times 100$$

The thickness of each shell was measured at 3 different points of the eggs using a micrometer (Mitutoyo 0.01–5 mm) by separating the membrane. Next, the assessment was carried out by taking the average of these three values for each egg.

The following formula was used in order to measure shell ratio and Haugh unit:

$$\textit{Shell ratio (\%)} = \frac{\textit{Weight of the shell}}{\textit{Weight of the egg}} \times 100$$

Haugh unit: $100\ log\ (H + 7.57 - 1.7\ W^{0.37})$

(Nesheim et al. 1979).

H: Height of the albumen, (mm)

W: Weight of the egg, (g)

Serum, tibia and excreta criteria

At the end of the 10 weeks, the serum was extracted from the blood supplied from 3 animals in each group and, serum total protein (TP), calcium (Ca) and P values were determined spectrophotometrically by the commercial kits.

At the end of the research, left tibias were taken from 4 animals representing each experimental groups for determine the tibia criteria. Muscles, cartilages, and membranes were cleared from the bones, the tibia weight was measured using a precision balance, and tibia length and diameter were measured with digital calliper. Then ash, Ca and P were analyzed in the tibia (AOAC, 2000). During the last two days of the egg period, excreta samples were taken from each of the 5 pens. Then these samples were dried, and levels of ash, Ca and P were determined in the samples (AOAC, 2000).

Statistical Analysis

The data obtained from the research was analysed by the method of "analysis of variance in experimental arrangement of completely randomized factorial design" (Düzgüneş et al., 1987). In determining the different groups, the "Duncan Multiple Comparison Test" was used (Duncan, 1955). The variance analyses were carried out by using Minitab 15.1, Duncan tests MStat – C statistical programs.

Results and Discussion

The average of feed consumption, feed conversion ratio and egg yield found from the experimental groups were shown in Table 3. The feed consumption of the quails was not affected neither enzymes supplementation nor different CP levels in their diets. This result had similarities with the research findings, stating that the phytase addition to laying hen diets did not affect the feed consumption (Casartelli et al., 2005; Liebert et al., 2005) and had differences with some studies stating that it increased feed consumption (Punna and Roland, 1997; 1999; Jalal and Scheideler, 2001; Francesch et al., 2005; Musapuor et al., 2005). During the research, the level of protein or enzymes did not have a significant effect on the feed conversion ratio ($P > 0.05$). The similar results were reached in both the studies of Bustany and Elwinger (1988), and Yörük and Bolat (2003). No differences were determined in terms of egg production in the experimental groups. While this result is consistent with some studies reporting that the addition of enzymes to barley-based feed given to laying hen does not affect the egg production (Bustany and Elwinger, 1988; Yörük and Bolat, 2003; Jalal and Scheideler, 2001), it was different from the studies reporting that phytase increased the egg production (Van der Klis et al., 1997; Punna and Roland, 1999; Sohail and Roland, 2000; Jalal and Scheideler, 2001; Çabuk et al., 2004; Francesch et al., 2005). The CP level did not affect the quail performance (Table 3). Similarly, it was reported by Tarasewich et al. (2006) that the decrease of the CP rate of quail rations during laying period from 21 % to 19 % did not have any negative effect on the animal performance. In order to provide the optimum performance, the CP amount of that is necessary to be found in layer quail ration was suggested in different ratios as 18 % (Murakami et al., 1993; Garcia et al., 2005; Abaza et al., 2009; Olgun and Yıldız, 2014), 20 % (NRC, 1994), 22.42 % (Pinto et al., 1998), and 21.95 % (Soares et al., 2003).

In this research, CP x phytase x β-glucanase interaction was found to be important in terms of egg weight (Table 4). The effect of the interaction was summarized in Table 5. Accordingly, the addition of only β-glucanase to rations, including 16% CP, increased the egg weight compared to the group without enzyme addition ($P < 0.05$). It was determined that the negative effect of phytase on the egg weight at the level of 16% and 18% CP disappeared when the ration CP level was increased to 20 %. These results taken in terms of egg weight contradict with the studies showing that the addition of phytase does not affect egg weight (Bustany and Elwinger, 1988; Musapuor et al., 2005). In this study, it was thought that the differences in the egg weight do not result from the enzyme factor added to the rations, but from the feed's CP content. Yet, no negative effect of phytase on the egg weight was seen in the groups with 20% CP content. Novak et al. (2006) also reported that the protein deficiency in the chicken diets caused the decrease in egg weight. In the recent years, in researches conducted by quails fed with 18% CP were found to be producing heavier eggs than the ones fed with 16% CP (Sangilimadan et al., 2012; Olgun and Yıldız, 2014).

During the research, it was found that addition of β-glucanase to the feed considerably increased the egg mass ($P<0.01$) (Table 4). Jaroni et al. (1999) reported that the addition of xylanase and protease to wheat based laying hen diets increased not only egg mass but also egg weight. This study showed that β-glucanase has no effect on the performance criteria such as feed consumption, feed conversion ratio and egg production, but the study also showed that in terms of egg mass, β-glucanase usage in barley-based laying quail rations is beneficiary.

Table 3. Effects of CP levels and enzyme supplementation on performance

Experimental groups[1]	Body weight (g/20th weeks)	Feed intake (g/bird per week)	FCR (g/g egg weight)	Egg production (%)
C16	241.01±3.83	218±4.51	2.50±0.045	78.92±3.20
F16	244.87±6.72	217±6.16	2.46±0.078	77.31±3.20
B16	240.48±6.16	222±7.25	2.44±0.099	81.36±4.98
FB16	245.17±4.50	222±7.26	2.54±0.079	87.36±1.19
C18	248.78±3.18	236±4.46	2.57±0.046	82.51±1.96
F18	239.55±4.65	223±7.95	2.58±0.072	80.87±2.07
B18	244.87±3.90	224±6.09	2.48±0.082	81.00±3.52
FB18	246.04±6.11	225±8.12	2.50±0.118	84.26±4.48
C20	247.25±3.61	232±4.38	2.57±0.056	81.58±2.91
F20	246.05±4.60	225±5.75	2.49±0.052	80.07±2.83
B20	251.75±3.90	223±7.66	2.44±0.072	80.38±3.28
FB20	245.55±4.43	226±4.40	2.41±0.051	83.69±5.82
P values				
CP	0.109	0.207	0.490	0.929
Phytase	0.723	0.434	0.979	0.529
β-glucanase	0.132	0.624	0.175	0.178
CP x phytase	0.812	0.819	0.775	0.954
CP x β-glucanase	0.705	0.515	0.598	0.498
Phytase x β-glucanase	0.261	0.211	0.349	0.165
CPxphytasexβ-glucanase	0.190	0.743	0.882	0.952

[1]C16: % 16 CP, with no added enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, with no added enzyme; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, with no added enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.

In this research, while it was observed that the CP level of the feed had no effect on egg mass, Aboul-Ela et al. (1992) reported that in Bobwhite quails, the egg mass of the animals fed with 18 – 21% CP is heavier than those fed with 12 – 15% CP. It was thought that, this difference results from the use lower level CP in the study performed by Aboul-Ela et al. (1992). The results of the egg quality criteria were given in Table 4. No difference was determined in terms of egg shape index, shell thickness, shell ratio and Haugh Unit. Yaghobfar et al. (2007) reported that in the laying hens fed with rations added β-glucanase and xylanase based on dehulled barley, Haugh Unit was not affected by enzyme addition but this study demonstrated a decrease in shell weight and shell thickness ($P<0.05$) in the laying hens.

The results of blood serum TP, Ca and P values were shown in Table 6. As CP level in the ration increased, the serum TP level increased as well ($P < 0.01$). This finding supports the results of Tuleun et al. (2013) who reported that there was a positive correlation between the protein consumed and the level of serum total protein. At the end of the research, it was determined that enzyme additions did not affect the serum TP, Ca and P levels. While El-Deeb et al. (2000) reported that the phytase addition did not affect the serum Ca level, Hassanien and Elnagar (2011) reported that it affected Ca and P levels. It was seen in the study by Hassanien and Elnagar (2011), this difference was due to the phytase usage in the diet over the level of 500 FTU kg^{-1}.

The results regarding the tibia criteria were given at Table 7. In terms of all studied criteria, no difference of statistical significance was seen between the groups. Um et al. (1999, reported that addition of phytase to the laying hen rations did not change the tibia weight, length and ash, but it increased amounts of Ca and P. In another research where phytase addition was 1000 FTU kg^{-1}, it was reported that the amounts of tibia ash and tibia P increased significantly (Musapour et al., 2005).

It was considered that the emerging different results in terms of tibia criteria arose from the amount of phytase used in the related research and available P levels in the rations. Because, the researchers obtained these results using lower available P and higher level of phytase.

The results of excreta ash, Ca and P contents of groups were shown in Table 8. While no difference was observed between the groups in terms of excreta ash, phytase added to the rations decreased the P excretion significantly ($P < 0.01$). This finding obtained in the research is consistent with the other studies carried out with laying hens (Francesch et al., 2005; Casartelli et al., 2005; Panda et al., 2005).

At the end of the analysis, it was determined that β-glucanase increased the Ca amount in the excreta ($P < 0.05$). This result contradicts the research of Juanpere et al. (2005) who reported that there was a positive interaction between the β-glucanase and phytase used in broiler rations, and that if these two enzymes were used together, the amount of Ca in excreta would decrease. As no research was found on β-glucanase's effect on mineral excretion in laying animals, no discussion could be done on this subject.

Table 4. Effects of CP level and enzyme supplementation on egg quality parameters

Experimental groups[1]	Egg weight (g)	Egg mass (g)	Shape index (%)	Shell thickness (mm)	Shell percentage	Haugh Unit
C16	12.45±0.168	9.83±0.430	76.38±0.447	0.23±0.002	8.15±0.118	84.65±0.852
F16	12.59±0.141	9.73±0.472	77.35±0.548	0.23±0.004	8.26±0.115	84.90±0.737
B16	13.02±0.178	10.59±0.366	76.72±1.190	0.23±0.002	8.13±0.117	84.64±0.549
FB16	12.51±0.113	10.93±0.180	75.64±0.737	0.23±0.006	7.77±0.254	83.34±0.746
C18	13.12±0.106	10.83±0.283	77.30±0.631	0.23±0.003	8.14±0.122	83.42±0.710
F18	12.34±0.237	9.98±0.316	77.04±0.711	0.22±0.003	8.28±0.099	84.11±1.180
B18	12.88±0.166	10.43±0.526	76.69±0.597	0.23±0.004	8.00±0.134	83.96±0.555
FB18	12.85±0.213	10.83±0.503	76.69±0.635	0.23±0.005	8.34±0.190	83.12±0.633
C20	12.92±0.132	10.54±0.542	75.16±0.426	0.23±0.003	8.31±0.085	82.79±0.612
F20	12.89±0.198	10.32±0.435	77.06±0.720	0.23±0.004	8.26±0.208	83.37±0.831
B20	13.04±0.171	10.48±0.427	76.05±0.928	0.23±0.004	8.38±0.108	83.33±0.895
FB20	13.38±0.098	11.19±0.103	77.18±1.390	0.24±0.004	8.21±0.216	85.10±1.200
β-glucanase effect						
No	-	10.26±0.162 B	-	-	-	-
Yes	-	10.94±0.167 A	-	-	-	-
P values						
CP	0.004	0.384	0.545	0.057	0.130	0.388
Phytase	0.154	0.722	0.309	0.893	0.989	0.702
β-glucanase	0.022	0.002**	0.612	0.931	0.274	0.933
CP x phytase	0.075	0.234	0.216	0.456	0.148	0.356
CP x β-glucanase	0.779	0.242	0.485	0.695	0.406	0.271
Phytase x β-glucanase	0.431	0.071	0.320	0.283	0.477	0.527
CPxphytasexβ-glucanase	0.013*	0.301	0.551	0.334	0.306	0.433

[1]C16: % 16 CP, with no added enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, with no added enzyme; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, with no added enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.
*(P<0.05), **(P<0.01).

Table 5. Effects of CP x phytase x β-glucanase on egg weight (g).

	Phytase			
	No		Yes	
	β-glucanase			
	No	Yes	No	Yes
CP level (%)				
16	12.45±0.168 Bb1	13.02±0.178 Aa1	12.59±0.141 Aab1	12.51±0.113 Ab2
18	13.12±0.106 Aa1	12.88±0.166 Aa1	12.34±0.237 Ab2	12.85±0.213 Ab1
20	12.92±0.132 Aa1	13.04±0.171 Aa1	12.89±0.198 Aa1	13.38±0.098 Aa1

[A,B] The same levels of crude protein and phytase with different capitalization levels of β-glucanase are significant (P<0.05).
[a,b] The same levels of crude protein and β-glucanase with different small letters levels of phytase are significant (P<0.05).
[1,2] The same levels of β-glucanase and phytase with different figures levels of crude protein are significant (P<0.05).

Table 6. Effects of CP levels and enzyme supplementation on serum TP, Ca and P levels

Experimental groups[1]	TP (g/dL)	P (mg/dL)	Ca (mg/dL)
C16	3.43±0.328	7.42±0.129	20.40±0.422
F16	3.88±0.105	8.06±0.144	21.05±0.899
B16	3.79±0.040	7.40±0.139	19.96±0.219
FB16	3.91±0.121	7.49±0.139	20.55±0.983
C18	3.84±0.188	7.45±0.068	20.17±0.493
F18	4.50±0.111	7.69±0.040	20.62±1.030
B18	3.88±0.051	7.21±0.444	20.67±0.567
FB18	3.94±0.192	7.21±0.712	19.71±0.125
C20	4.45±0.261	7.38±0.170	20.41±0.331
F20	4.46±0.257	7.76±0.383	19.91±0.176
B20	4.57±0.166	7.35±0.555	19.83±0.605
FB20	4.32±0.301	7.49±0.151	19.72±0.382
CP effect			
16	3.69±0.139 C	7.56±0.093	20.47±0.294
18	3.99±0.106 B	7.40±0.152	20.27±0.289
20	4.45±0.124 A	7.47±0.139	20.06±0.193
P values			
CP	0.003**	0.453	0.595
Phytase	0.257	0.950	0.134
β-glucanase	0.859	0.301	0.110
CP x phytase	0.391	0.466	0.821
CP x β-glucanase	0.480	0.948	0.866
Phytase x β-glucanase	0.199	0.600	0.297
CPxphytasexβ-glucanase	0.893	0.534	0.907

[1]C16: % 16 CP, with no added enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, with no added enzyme; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, with no added enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.
** (P<0.01).

Table 7. Effects of CP levels and enzyme supplementation on some tibia characteristics.

Experimental Groups[1]	Drymatter (%)	Weight (%)	Length (mm)	Ash (%)	Ca (ash, %)	P (ash, %)
C16	93.26±0.111	0.277±0.008	32.88±0.357	44.94±1.730	34.09±0.540	16.53±0.134
F16	92.65±0.377	0.269±0.018	32.88±0.434	44.67±1.610	34.65±1.780	16.53±0.116
B16	92.83±0.247	0.265±0.016	32.36±0.461	47.37±2.140	35.67±1.690	16.43±0.123
FB16	92.66±0.217	0.270±0.007	32.63±0.626	48.02±0.610	34.53±1.110	16.31±0.200
C18	92.78±0.136	0.278±0.009	32.63±0.439	45.19±1.040	34.14±0.894	16.53±0.324
F18	92.82±0.240	0.270±0.019	33.72±0.832	46.42±1.920	32.45±0.250	16.48±0.356
B18	92.77±0.061	0.281±0.002	33.30±0.377	46.44±1.140	33.57±0.658	16.22±0.220
FB18	92.58±0.566	0.264±0.019	33.37±0.618	44.59±1.900	34.35±1.260	15.83±0.760
C20	92.46±0.254	0.262±0.008	32.26±0.412	47.63±1.330	32.88±0.816	16.45±0.119
F20	92.62±0.129	0.282±0.020	33.06±0.708	46.61±1.020	34.06±1.830	15.82±0.395
B20	92.67±0.065	0.258±0.008	32.11±0.679	45.62±1.050	32.70±0.703	16.64±1.177
FB20	92.88±0.298	0.255±0.015	32.55±0.447	49.28±1.620	33.75±1.660	16.49±0.109
P values						
CP	0.562	0.497	0.134	0.366	0.207	0.690
Phytase	0.524	0.748	0.165	0.670	0.858	0.207
β-glucanase	0.838	0.286	0.555	0.302	0.567	0.684
CP x phytase	0.282	0.510	0.783	0.768	0.570	0.743
CP x β-glucanase	0.430	0.787	0.742	0.349	0.800	0.104
Phytase x β-glucanase	0.764	0.748	0.557	0.656	0.873	0.971
CPxphytasexβ-glucanase	0.645	0.711	0.707	0.249	0.439	0.614

[1]C16: % 16 CP, with no added enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase;
FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, with no added enzyme; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, with no added enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.

Table 8. Effects of CP levels and enzyme supplementation on excreta ash, Ca and P levels (%).

Experimental Groups[1]	Ash	Ca	P
C16	22.15±0.974	1.055±0.043	0.23±0.019
F16	20.79±0.910	1.097±0.117	0.17±0.008
B16	24.45±2.310	1.189±0.079	0.18±0.011
FB16	20.93±0.404	1.154±0.126	0.14±0.009
C18	21.72±1.080	1.173±0.096	0.19±0.014
F18	22.74±1.010	0.988±0.072	0.15±0.009
B18	21.26±1.380	1.305±0.054	0.17±0.002
FB18	20.76±0.566	1.135±0.057	0.16±0.008
C20	20.07±0.516	1.042±0.052	0.19±0.011
F20	20.33±0.867	0.908±0.029	0.16±0.008
B20	19.97±0.484	1.076±0.064	0.19±0.020
FB20	21.36±0.422	1.115±0.102	0.20±0.011
β-glucanase effect			
No	21.31±0.393	1.057±0.030 b	0.19±0.007
Yes	21.46±0.506	1.162±0.034 a	0.17±0.005
Phytase effect			
No	21.51±0.474	1.122±0.029	0.19±0.006 A
Yes	21.15±0.309	1.066±0.037	0.16±0.004 B
P values			
CP	0.097	0.121	0.214
Phytase	0.477	0.120	0.002**
β-glucanase	0.807	0.015*	0.359
CP x phytase	0.086	0.281	0.232
CP x β-glucanase	0.277	0.932	0.057
Phytase x β-glucanase	0.500	0.697	0.086
CPxphytasexβ-glucanase	0.531	0.553	0.868

[1]C16: % 16 CP, with no added enzyme; F16: % 16 CP, with added phytase; B16: % 16 CP, with added β-glucanase; FB16: % 16 CP, with added phytase+β-glucanase, C18: % 18 CP, with no added enzyme; F18: % 18 CP, with added phytase; B18: % 18 CP, with added β-glucanase; FB18: % 18 CP, with added phytase+β-glucanase; C20: % 20 CP, with no added enzyme; F20: % 20 CP, with added phytase; B20: % 20 CP, with added β-glucanase; FB20: % 20 CP, with added phytase+β-glucanase.
*(P<0.05), ** (P<0.01).

It is concluded that CP level of barley based quail rations affected the weight of the egg, and for this reason the rate of the protein should not be lower than 18 % during this period. It has been determined that β-glucanase addition had a positive effect on egg quality because of the egg mass. Furthermore, the addition of phytase to poultry diets could be a suitable method for decreasing P excretion, and thus preventing environmental pollution.

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