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EFFECTS OF PHYTASE AND DCP SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, SOME SERUM, TIBIA AND EXCRETA CHARACTERISTICS OF BARLEY BASED PROTEIN DEFICIENT QUAIL DIETS

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This research was conducted to determine the effects of phytase or dicalcium phosphate (DCP) supplementation on performance, egg quality, serum total protein (TP), calcium (Ca) and phosphorus (P) level, tibia ash, Ca and P concentration, excreta ash, Ca and P level of barley based protein deficient quail diets. A total of 270, 10 wk old quails were used. Diets were insufficient in available P and had a different crude protein (CP) level (16, 18, 20%). It was made phytase or DCP supplementation on each protein level. At the end of the study, quails had similar body weight, body weight change and feed conversion ratio although feed consumption was affected by the CP level ($P<0.05$). Egg weight was heaviest in 18, 20% CP level ($P<0.05$). Albumen index, Haugh Unit, serum TP and excreta P level ($P<0.05$) were affected by CP percentages of the diet. Phytase increased the serum P concentration ($P<0.05$). P excretion was lowest in control and phytase groups by 38% ($P<0.01$). As a result, the CP level of quail diets shouldn't be lower than 18% and DCP didn't cause any improvement of the research criteria in the layer period. Phytase can be used in this period if it is economical.

Key words: barley; DCP; phytase; protein; quail

ВЛИЈАНИЕ НА ДОДАВАЊЕТО ФИТАЗА И ДКФ ВО ХРАНАТА ЗА ПРЕПЕЛИЦИ СОСТАВЕНА ОД ЈАЧМЕН СО ПОНИСКА СОДРЖИНА ПРОТЕИНИ ВРЗ ПЕРФОРМАНСИТЕ, КВАЛИТЕТОТ НА ЈАЈЦАТА, НЕКОИ КАРАКТЕРИСТИКИ НА СЕРУМОТ, ТИБИЈАТА И ЕКСКРЕТИТЕ

Целта на истражувањата беше да се утврди влијанието на додавање фитаза или дикалциумфосфат (ДКФ) во храната за препелици составена од јачмен со пониска содржина протеини врз перформансите, квалитетот на јајцата, вкупните протеини во серумот (ВП), нивото на калциум (Ca) и фосфор (P), пепелот во тибијата, концентрацијата на Ca и P во тибијата и пепелот во екскретот и концентрацијата на Ca и P во екскретот. Беа користени вкупно 270 препелици на возраст од 10 недели. Храната содржеше намалено количество искористлив фосфор, а застапеноста на суровите протеини беше различна (16%, 18% и 20%). Фитазата и ДКФ беа додавани во храната која содржи различен процент протеини. На крајот од истражувањето, препелиците имаа слична жива маса, промената на живата маса и односот помеѓу конверзијата на храна и консумацијата на храна беа под влијание на нивото на суровите протеини ($P<0,05$). Масата на јајцата беше поголема при користење на храна која содржи 18% и 20% протеини ($P<0,05$). Индексот на албумен, Хофовите единици, вкупните протеини во серумот и нивото на фосфор во екскретот беа под влијание на содржината на суровите протеини во храната. Фитазата ја зголеми концентрацијата на фосфор во серумот ($P<0,05$). Екскрецијата на фосфорот беше најмала кај контролната група и кај групата со фитаза (38%) ($P<0,01$). Врз основа на резултатите беше заклучено дека нивото на сурови протеини во храната за препелици не треба да биде пониско од 18%. Додавањето на ДКФ не придонесува за подобрување на испитуваните критериуми во периодот на несење. Фитазата може да се користи во овој период доколку тоа е економично.

Клучни зборови: јачмен; ДКФ; фитаза; протеини; препелици.

INTRODUCTION

Phosphorus (P) is one of the most essential and expensive macrominerals for the living organism. The majority of P (approximately two-thirds) in the feedstuffs of poultry is contained in the chemical structure of phytic acid or its salts, which are known as phytates (Pallauf and Rimbach, 1997). The NRC (1994), nonphytate phosphorus (nPP) recommendation for the layer Japanese quail was 0.35%. For this reason, diets are usually supplemented with an inorganic P, generally dicalcium phosphate (DCP). The world is now faced with a P crisis driven by the declining global reserves of rock phosphate (Abelson, 1999). Furthermore, excess P is excreted and can lead to environmental damage. In recent years, one of the most common methods of reducing phosphorus excretion is the supplementation of poultry feed with phytase. Phytase hydrolyzes phytate phosphorus thus the addition of inorganic P can be decreased to make economical and environment friendly production.

Several reports have indicated that in addition to improving P availability, the availabilities of a number of amino acids to poultry can be increased by the addition of phytase to the diet (Yi et al., 1996; Sebastian et al., 1997; Namkung and Leeson, 1999; Ravindran, 1999). Because dietary nitrogen (N) intake affects nitrogen content in manure, diet management has been recognized as a means to reduce ammonia emissions from poultry operations (Latshaw and Zhao, 2011). In addition to reducetions in inorganic P supplementation, phytase increases in protein-amino acid utilization would also enable producers to feed lower-protein diets and reduce N excretion, thereby creating both economic and environmental advantages.

The first aim of this research was to evaluate the influence of phytase or DCP supplementation on performance, egg quality, serum total protein (TP), calcium (Ca) and phosphorus (P) level, tibia and excreta ash, Ca and P concentration of barley based quail diets, the second one was to determine the interaction with phytase and protein and also find the optimum crude protein (CP) level of layer diets which did not negatively affect the birds performance, egg production and quality.

MATERIAL AND METHODS

Two hundred seventy Japanese quails were located randomly to nine experimental diets at ten replicates (3 birds in each cage) and fed from 10 to 20 weeks of age. The quails were fed equal daily amount of essential amino acids in barley-soybean meal diets that provided 16, 18 or 20% CP and a calculated metabolisable energy (ME) level of 2900 kcal/kg. The experimental diets were arranged by NRC (1994) recommendations except for P and CP content. Control diets (0.19–0.24% nPP) were used, and supplemented the control diet with exogenous phytase (500 FTU kg^{-1} of phytase) or DCP in each protein level (Table 1). The microbial phytase (CBS 517.94), used in this study was a Peniophora lycii product (Rovaphos-Trouw Nutrition). Quails were fed mash form feed ad-libitum and given free access to water. Feed samples were analyzed as described by AOAC (1990).

Feed consumption and body weight gain (determined individually) of chicks were recorded and feed conversion ratio (FCR) calculated weekly. Egg production and weight were determined daily and biweekly, respectively. At the end of the experiment (20 weeks of age), four quails were selected randomly from each group and killed by cervical dislocation to removed left tibia and blood samples were collected in 3 of them. Sera were separated and then stored at –20 until assayed. Concentrations of TP, Ca and P level of serum were measured by a commercial kit in a spectrophotometer. Tibia ash, Ca and P content of dried, fat free tibia bone were detected as described by AOAC (1990), after determination of tibia physical characteristics such as weight, width, length. An experiment completion, a 2 day, 5 excreta samples were collected in each group to find the level of ash, Ca and P (AOAC, 1990). All research data were analyzed by the two way ANOVA procedure using Minitab 15 software for the analysis of variance as a completely factorial design. Significant differences among treatments were identified by Duncan's multiple range tests (Duncan, 1955).

RESULTS

Body Weight (BW), Body Weight Change (BWC), Feed Intake (FI) and Feed Conversion Ratio (FCR)

There were no interactions between P and CP levels of diet in respect to all research criteria.

The mean values of BW, BWC, FI and FCR were presented in Table 2. Neither phytase not DCP affected BW, BWC, FI and FCR. However CP level of diet was significantly affected by FI ($P<0.05$). The lower FI was observed in 16% CP as compared with the other groups.

Table 1

Ingredients and nutrient composition of the experimental diets

Feeds,%	**C16**	**F16**	**DCP16**	**C18**	**F18**	**DCP18**	**C20**	**F20**	**DCP20**
Barley	49.00	49.46	48.00	45.10	45.05	45.55	41.00	41.00	41.00
Corn	19.90	19.40	19.80	18.00	18.00	18.00	17.67	17.61	17.70
Soybean meal	17.54	17.50	18.00	23.30	23.30	21.40	26.60	26.60	25.70
Fish meal	1.00	1.00	1.00	1.00	1.00	2.20	2.40	2.40	3.00
Sunflower oil	5.05	5.07	5.28	5.38	5.37	5.39	5.40	5.40	5.45
Limestone	6.29	6.29	5.71	6.27	6.27	5.70	6.11	6.11	5.70
DCP	–	–	1.00	–	–	0.82	–	–	0.63
DL-Methionine	0.19	0.19	0.19	0.16	0.16	0.15	0.12	0.12	0.12
L-Lysine	0.19	0.19	0.18	0.03	0.03	0.03	–	–	–
L-Treonine	0.14	0.14	0.14	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
Vitamin mixture[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral mixture[2]	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	–
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Calculated and analyzed nutrient values									
ME, kcal/kg[3]	2849	2845	2850	2849	2846	2850	2848	2845	2850
Crude protein,%[4]	16.09	16.02	16.10	18.10	18.09	18.08	20.00	20.10	20.06
Crude fat,%[4]	7.20	7.20	7.40	7.50	7.50	7.50	7.50	7.50	7.50
Crude fibre,%[4]	2.90	2.90	2.87	3.00	3.00	2.97	3.10	3.10	3.00
Crude ash,%[4]	9.00	9.00	9.40	9.20	9.20	9.40	9.40	9.40	9.40
Calcium[4]	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Total phosphorus,%[4]	0.38	0.38	0.39	0.40	0.40	0.43	0.46	0.46	0.47
nPP[3]	0.19	0.19	0.35	0.20	0.20	0.35	0.24	0.24	0.35
Methonine[5]	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Met+sistin[5]	0.73	0.73	0.73	0.76	0.76	0.76	0.78	0.78	0.78
Lysine[5]	1.00	1.00	1.00	1.00	1.00	1.00	1.12	1.12	1.12
Arinine[5]	1.00	1.00	1.00	1.15	1.15	1.15	1.30	1.30	1.30
Threonine[5]	0.74	0.74	0.74	0.74	0.74	0.74	0.77	0.77	0.77
Linoleic acit[5]	4.20	4.20	4.40	4.40	4.40	4.40	4.40	4.40	4.40

Abbreviations: C16: Control with 16% CP; F16: Phytase with 16% CP; DCP16: DCP with 16% CP; C18: Control with 18% CP; F18: Phytase with 18% CP; DCP18: DCP with 18% CP; C20: Control with 20% CP; F20: Phytase with 20% CP; DCP20: DCP with 20% CP.

[1]Provided per kg of premix; 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-pantotenate 12.000 mg, vit B_6 4000 mg, vit B_{12} 15 mg, folic acid 1000 mg, biotin 45 mg, choline chlorid 125.000 mg

[2]Provided per kg of mineral premix; 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.

[3]Calculated analyses.

[4]Determined analyses.

[5]Based on NRC (1994)

Egg production and quality

The effect of treatments on egg production and some egg quality parameters are given in Tables 2–3. There were no significant differences in egg production, egg mass, shell thickness, shell weight, albumen height, and cracked/broken eggs ratio although the egg weight was found lowest in 16% CP (P<0.05). Controversially the highest albumen index and the Haugh unit were recorded in 16% CP, as compared with the other groups (P<0.05).

Table 2

Mean performance value of Japanese quails influenced by dietary levels of P and crude protein

Groups	Initial BW (g)	10^{th} w BW (g)	BWC (1–10 w) (g)	FI (1–10 w) (g/b/w)	FCR (1–10 w) (g/g)	Egg prod. (1–10 w) (%/b/d)
			P effect			
Control	234.8±2.66	244.0±2.83	10.13±2.21	227.7±4.00	2.54±0.04	81.19±2.43
Phytase	230.8±2.48	243.6±3.07	11.77±2.58	221.5±3.72	2.51±0.03	79.37±1.58
DCP	235.5±2.86	247.2±3.02	11.23±2.06	229.68±3.74	2.57±0.04	80.77±2.08
			CP effect			
16 CP	234.9±2.89	242.2±3.36	8.71±2.83	$218.0±3.58^b$	2.54±0.041	78.38±2.35
18 CP	230.8±2.37	245.7±2.66	13.38±2.10	$231.8±4.06^a$	2.51±0.039	81.98±1.47
20 CP	235.3±2.72	246.9±2.84	11.18±1.83	$229.6±3.50^a$	2.57±0.040	81.08±2.13

[a, b] Mean values within a column indicated with different superscripts are significantly different (p<0.05)
BW: Body weight, BWC: Body weight change, FI: Feed intake, FCR: Feed conversion ratio

Table 3

Mean egg quality of Japanese quails influenced by dietary levels of P and crude protein

Groups	Egg weight (g)	Egg mass (g/b/d)	Shell thickness (mm)	Shell weight (g)	Albumen height (mm)	Albumen index (%)	Haugh unit	Broken egg (% total egg)
				P effect				
Control	12.85±0.127	10.39±0.300	0.23±0.002	1.07±0.013	3.62±0.073	4.88±1.20	83.03±0.497	3.54±0.940
Phytase	12.62±0.115	10.03±0.238	0.23±0.002	1.06±0.010	3.70±0.070	5.10±1.13	83.71±0.422	4.41±0.809
DCP	12.80±0.119	10.37±0.301	0.23±0.002	1.07±0.013	3.66±0.070	5.04±1.21	83.17±0.485	2.28±0.485
				CP effect				
16 CP	$12.50±0.120^b$	9.82±0.322	0.23±0.001	1.05±0.013	3.77±0.060	$5.22±0.96^a$	$84.20±0.405^a$	4.77±1.000
18 CP	$12.88±0.122^a$	10.54±0.227	0.23±0.002	1.07±0.012	3.54±0.070	$4.82±1.18^b$	$82.36±0.485^b$	2.83±0.497
20 CP	$12.91±0.108^a$	10.45±0.266	0.23±0.002	1.08±0.012	3.67±0.075	$4.98±1.29^{ab}$	$83.32±0.459^{ab}$	2.55±0.656

[a, b] Mean values within a column indicated with different superscripts are significantly different (p<0.05)

Tibia parameters

There weren't found any differences of tibia physical characteristics, ash content and Ca, P percentage of tibia. These findings are summarized in Table 4. Tibia length, width, dry matter content, ash level, Ca and P content of tibia ash didn't effect any experimental procedure through the study (P>0.05).

Table 4

The effect of treatments on tibia characteristics

Groups	Tibia length (mm)	Tibia width (mm)	Tibia DM (%)	Tibia ash (%)	Tibia P (% ash)	Tibia Ca (% ash)
			P effect			
Control	32.9±0.287	3.16±0.040	92.95±0.184	45.74±1.12	16.42±0.148	32.92±0.578
Phytase	33.2±0.371	3.11±0.052	92.69±0.143	45.90±0.86	16.28±0.191	33.72±0.824
DCP	32.3±0.347	3.17±0.037	92.72±0.155	46.09±1.22	16.59±0.187	34.50±0.600
			CP effect			
16 CP	32.89±0.267	3.19±0.042	93.06±0.161	44.85±1.23	16.53±0.093	34.28±0.648
18 CP	33.00±0.410	3.14±0.047	92.79±0.114	45.60±0.91	16.52±0.237	33.58±0.634
20 CP	32.53±0.361	3.12±0.040	92.51±0.171	47.29±0.93	16.24±0.168	33.28±0.785

Serum TP, Ca and P level

In the layer period, phytase supplementation on barley based quail diets had a significant effect on the serum P level (P<0.05) (Table 5). The lowest mean value was determined 7.31 mg/dl in the control group. The CP level didn't effect the serum P content although the CP content of diets affected the serum TP level, the highest value was found in the group of 20% of CP (P<0.05). The serum Ca level didn't change any research treatment (P>0.05).

Table 5

The effect of treatments on some serum and excreta parameters

Groups	Serum TP (g/dl)	Serum P (mg/dl)	Serum Ca (mg/dl)	Excreta ash (%)	Excreta P (% ash)	Excreta Ca (% ash)
			P effect			
Control	3.73±0.271	7.31±0.059^{b}	20.35±0.331	22.38±0.818	0.74±0.043^{B}	5.22±0.248
Phytase	4.28±0.132	7.84±0.132^{a}	20.53±0.431	21.29±0.570	0.75±0.035^{B}	4.79±0.235
DCP	4.09±0.225	7.52±0.122^{ab}	20.31±0.431	20.24±0.548	1.19±0.034^{A}	5.08±0.190
			CP effect			
16 CP	3.58±0.227^{b}	7.63±0.142	20.62±0.391	21.70±0.717	0.95±0.068^{a}	5.10±0.194
18 CP	4.06±0.167^{ab}	7.53±0.060	20.32±0.442	22.06±0.783	0.82±0.075^{b}	5.03±0.308
20 CP	4.46±0.184+	7.51±0.168	20.24±0.235	20.16±0.432	0.91±0.053^{ab}	4.95±0.184

$^{A, B}$ Mean values within a column indicated with different superscripts are significantly different (p<0.01).
$^{a, b}$ Mean values within a column indicated with different superscripts are significantly different (p<0.05).

Excreta ash, Ca, P level

Excreta results are presented in Table 5. Phytase or DCP supplementation on barley based which has a different CP level of quail diets didn't affect the excreta ash content. P excretion was affected by phytase supplementation of diet. The lowest P excretion was in Phytase and the control groups (P<0.01). Interestingly the CP level of diet also changed the P excretion (P<0.05) and the lowest excretion of 18% CP than the other groups.

DISCUSSION

In this research phytase didn't affect BW, BWC, FI and FCR. These results are with conflicting Carlos and Edwards (1998), Jalal and Scheideler (2001), Fassani et al. (2011), who reported that the addition of phytase significantly improved BW, FI and FCR, and only FCR, respectively. On the other hand Fassani et al. (2011) reported that phytase didn't change FI. Van der Klis (1997) noticed that the body growth and the tibia ash of laying hens can be positively affected by supplementing phytase when the diets contain 0.12% nPP. Similar results were found by Panda et al. (2005). In this research phytase did not effect the performance parameters. These findings may be attributed to the nPP level of our experimental diets (0.19% min.). In another research no differences were found when 0.18% nPP diets with no phytase supplements were compared to 0% nPP diets and 1,000 FTU/kg phytase (Narahari and Jayaprasad, 2000).

In another way, FI was affected by the CP level of the experimental diet. Feed consumption decreased as protein intake decreased ($P<0.05$). This result was similar to those obtained by Novak et al. (2006).

In our study, feed supplementation with phytase had no effect ($P>0.05$) on the egg production and the egg weight, as the same results were found by Carlos and Edwards (1998), Jalal and Scheideler (2001). Similarly, there were no significant differences in the egg mass, shell thickness, shell weight, albumen height, albumen index, Haugh unit and broken eggs. Some authors recorded that the egg shell quality and the broken egg to total egg ratio were not different among treatments in agreement with our results (Van der Klis et al., 1997; Um and Paik, 1999; Lim et al., 2003; Kim et al., 2005). But Jalal and Scheideler (2001) determined that supplemental phytase increased the egg mass.

The CP level of barley based quail diet changed the egg weight significantly ($P<0.05$). The highest value occurred in 18 – 20% CP. This result confirmed that Novak et al. (2006) and Murakami and Furlan (2002) reported that the egg size depends greatly on the daily crude protein intake, since layers do not store large amounts of protein.

The albumen index and the Haugh unit were increased by decreasing dietary protein. Leeson and Caston (1997), Novak et al. (2006) reported similar responses as ours for Haugh units when feeding low protein diets. In contrast, Hamilton (1978) observed no significant change in Haugh units when feeding low protein diets to laying hens. In another way, we didn't meet any research about the albumen index and the CP level interaction so it is needed to perform further study on this subject.

Experimental treatments didn't change any tibia characteristics but Osman et al. (2009), demonstrated that phytase supplementation increased bone mineralization that led to increasing the bone rigidity in Japanese quail chicks fed with low nPP diets. This finding may be attributed to the age of our animals because of their immature quail chicks. In another study, investigators obtained that low dietary nPP (below 1.3 g/kg) was not able to support the optimum performance of hens during the laying cycle (from 22 to 46 weeks of age), either in maize or barley diets. The adverse effects of a low P diet were more severe in hens on a maize diet than in those on a barley diet. But groups which consumed diet containing phytase tibia ash percentage increased significantly (Francesch et al. 2005).

Phytase supplementation had a significant effect on the serum P content. This conclusion was supported by Carlos and Edwards (1998) who determined that phytase supplementation on layer diets increased the serum P level.

At the end of the experiment, significant reduction of P excretion can also be achieved. For Um and Paik (1999), the reduction of P excretion was 41% with the low P diet and supplementary phytase. Keshavarz (2000) reported that supplemental phytase could lower by more than 34–47% of P excretion by increasing P availability. In our research, P excretion was decreased by 38% and DCP groups were lower than it was reported by Boling et al. (2000) (app. 50%).

Studies have shown that microbial phytase improved availability of phytate phosphorus layer diets. However, little research is available concerning the influence of phytase on availability of Ca. In this study, tibia, serum and excreta Ca content weren't influenced by any research treatment. We suppose that, laying quails had enough of Ca level of diets' so they didn't need more.

Murakami et al. (1993) and Pereira et al. (2000) reported better performance of laying quails when the birds were fed 18% CP, similar to that found in our study. NRC (1994) recommended 20% CP, however, Pinto et al. (1998) found levels of protein requirements (22.42%), Singh and Narayan (2002) also reported 22% protein for quails in the production period. A higher level of protein requirement was noticed by Vilar et al. (1991), who observed higher egg production when the quails were fed 24% CP in the diet. All of these CP values were much higher than those found in this study.

CONCLUSION

Our findings showed that, phytase or DCP didn't provide any improvement on quail performance, egg quality and tibia characteristics if the diet nPP level was higher than 0.19%. There was no interaction between P and CP level of diet. The CP level of barley based quail diets shouldn't be lower than 18% for maintaining optimum egg weight and quality.

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