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Effect of Different Levels of Calcium, Phosphorus and Vitamin D_3 on the Calcium, Phosphorus and Magnesium of Plasma, Hatchability and Performance on the Boiler Breeder Hens

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Abstract: This experiment was carried out to effect of different levels of calcium, phosphorus and vitamin D_3 on the calcium, phosphorus and magnesium of plasma, hatchability and performance from Ross 308 broiler breeders with 20 weeks af age at begining of the experiment. A completely randomized experimental design was applied in 3×2 factorial arrangement with 3 levels of calcium 3.122, 85, 2.56 and 3 levels of phosphorous 0.41, 0.37, 0.33 and 2 levels of vitamin D_3 (3300, 3000 IU) with 4 replicates of 10 birds plus one cockerel in each pen. At end of experiment different parameters such as calcium, phosphorus and magnesium of plasma percentage, egg shell percentage, egg weight, calcium and phosphorus of tibia, hatchability, production and feed conversion ratio measured. After statistical analysis, it became clear that the treatments effect on all parameters except, production, hatchability and FCR cannot have a significant affect ($p<0.05$). It was concluded that the positive function of vitamin D_3 Iin this field.

Key words: Calcium, phosphorus, vitamin D_3, broiler breeder hens hatchability, significant effect, Iran

INTRODUCTION

Poor egg shell quality accounts for major economic losses to commercial egg producers. There are numerous factors involved in egg shell formation and its subsequent quality. The macro factors include but are not limited to the source and level of calcium in the diet, phosphorus level in the diet and temporal intake of these minerals. The source and particle size of calcium used in laying hen diets are 2 factors that have received considerable attention in recent years.

The egg shell must be strong enough to resist the processes of lay, collection, grading and transport reaching the final consumer intact. Egg shell defects include cracks, deformities and irregular calcium deposition in addition to invisible microbial contamination, resulting in economic losses. Modern layer breeds have high egg production and low body weight in the 1st laying cycle. Second-cycle layers produce large and extra large eggs and present a higher percentage of thin egg shells due to disorders associated to calcium and vitamin D_3 metabolism. Laying birds have high calcium requirements for bone maintenance and egg shell deposition which are supplied by adequate and available calcium dietary sources (Pizzolante *et al*., 2009). Phosphorus is an essential mineral for laying hens in the formation of egg shell and metabolism (Wu *et al*., 2006). Studies on optimal calcium levels in layer diets are economically important contributing to minimize broken egg shell percentage, production costs and environmental impacts (Oliveira *et al*., 2002). Calcium is used for bone formation, egg shell production and blood clotting. It also affects the heard, muscles and nerves as well as some of the body's enzyme systems. Most of the body's Ca is found in the skeleton, calcium is comprised mainly of calcium phosphate with some calcium carbonate. The shell deposition and shell quality are directly related to the calcium level in the diet.

The main mechanism by which vitamin D facilitates calcification of bone and formation of egg shell is believed to be a result of the effects of the physiologically active form of vitamin D, 1.25-Dhydroxycholecalciferol (1.25 (OH) D) on intestinal function. It is well established that in laying hens a vitamin D_3 dependent Ca-binding protein is involved in the active transport of Ca across the intestinal membrane and probably across the uterine membrane (Bolukbasi *et al*., 2005). Dietary Phosphorus (P) levels influence Ca metabolism in hens with the indication

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that growing birds respond to P deficiency by an increase in intestinal 1.2-dihydroxy vitamin D_3, Ca-Binding Protein (CaBP) and intestinal Ca absorption. These reasches suggest that low blood P stimulates the synthesis of 1,25-$(OH)_2D_3$ which is involved in Ca homeostasis. The most active form of vitamin D, 1,25-$(OH)_2D$ is responsible for increasing the absorption of intestinal Ca and phosphate, mobilizing these ions from bone and increasing bone mineralization (Orban *et al*., 1992).

The researchers conducted the present study to determine the effects of oviposition time, hen's age and extra dietary calcium on Egg Weight (EW), SG and EW loss during the first 18 days of incubation, fertility, embryo viability, age of embryonic death in fertile eggs and hatchability of eggs from feed-restricted broiler breeder hens under commercial conditions (Novo *et al*., 1997). Even though, the understanding of the importance of timing of Ca intake in maintaining maximum shell quality in broiler breeders is incomplete, it is unquestionable that shell quality is related to the ability of the hen to absorb Ca from the intestine and to utilize skeletal Ca (Reis and Fieo, 1995). It has long been known that vitamin D is needed for proper absorption of calcium (Fritts and Waldroup, 2003). Calcium and phosphorous are essential macro minerals with calcium forming a significant component of the shell and phosphorous playing an important role in skeletal calcium deposition and subsequent availability of calcium for egg shell formation during the dark period.

However, the feeding of calcium levels before the requirement of the bird for production has not been shown to improve shell quality. Indeed, feeding hens high levels of calcium may interfere with the availability of other minerals (NRC, 1994) and can have a negative impact on the ability of the bird to utilise calcium, particularly if calcium levels in the diet are subsequently decreased (Gerber, 2006). There are many various factor effect on the egg weight and egg size. Methionine, linoleic acid and supplemental fat are 3 factors that affect egg size (Safaa *et al*., 2008; Keshavarz, 2003).

MATERIALS AND METHODS

About 200 and 40 Ross 308 broiler breeder hens were used in this experiment which were 20 weeks of age and continued for 50 weeks periods. Birds were distributed in 24 pens (1.00 m long×1.00 width) with housing 10 birds per pen plus a cockerel each pen. Each cage was equipped with a cup drinker and a trough feeder place in front of the cage. A completely randomized experimental design was applied in 3×2 factorial arrangement that there were 4 experimental units for each of the 6 treatment groups. Diets were formulated to meet the nutrient requirements for Ross 308 broiler breeder (Table 1). All diets were isocaloric and isonitrogenous. There were six dietary treatments (one control and 5 experimental groups). Six experimental dietary include different level of calcium, phosphorus and vitamin D_3 in each treat. Control diet (T_3) containing about 15% CP, 2750 kcal ME kg^{-1}, (2.75, 0.37%) Ca, P and 3000 (IU) Vitamin D_3. In this experiment, use 2 levels of calcium, phosphorus (2.56, 0.33%) and (3.13, 0.4%) 10% lower and 10% higher of Ross 308 requirements, respectively.

Table 1: Ingredient and calculated composition of experimental diet

Ingredient/groups	T_1	T_2	T_3	T_4	T_5	T_6
Corn	34.33	34.33	35.67	35.67	35.87	35.87
Wheat	29	29	29.22	29.22	30.03	30.03
Wheat brain	6.69	6.69	6.75	6.75	6.81	6.81
Soybean meal	12.8	12.80	12.92	12.92	13.08	13.08
Rap seed	5.94	5.94	6	6	6.06	6.06
Ca $(Co_3)_2$	7.74	7.74	6.22	6.22	5.1	5.1
Monocalcium phosphate	1.2	1.20	0.915	0.915	0.742	0.742
Vitamin + Min premix	0.6	0.6	0.6	0.6	0.6	0.6
Salt	0.4	0.4	0.4	0.4	0.4	0.4
Methionine	0.8	0.8	0.8	0.8	0.8	0.8
Lysine	0.2	0.2	0.2	0.2	0.2	0.2
Oil	1.2	1.2	1.2	1.2	1.2	1.2
Vitamin D_3	3000	3300	3000	3300	3000	3300
Calculated ana1ysis						
ME Kcal kg^{-1}	2750	2750	2750	2750	2750	2750
Crude protein	15	15	15	15	15	15
Total Ca (%)	3.135	3.135	2.85	2.85	2.565	2.565
Availible P (%)	0.407	0.407	0.37	0.37	0.333	0.333

Vitamin D_3 have 2 level 3000 and 3300 (IU) in the experimental diets. At the end of the 50 weeks feeding period, blood samples were obtained from the brachial veins of 4 hens per treatment, the plasma was separated by centrifugation blood for 10 min at 2000 x g and saved for determination of plasma Ca, P and Mg. The Ca, P and Mg were measured on auto analyzer by using commercial kits. At end of experiment, 2 hens slaughtered per each pen for tibia (Ca, P) analyze.

The weights of the birds were recorded at beginning and end of the experimental period. During the experimental period, daily feed intake per bird egg weight, hatchability, egg production, egg shell losses, broken egg, total egg shell losses percentage and mortality were recorded. Feed conversion was calculated from feed intake to egg weight production. Statistical analyses was performed by the statistical package SPSS (1999) for windows, version 10.0. Multiple comparisons of the data were done by using the Duncan's test after One-Way Analysis of Variance (one-way ANOVA).

RESULTS AND DISCUSSION

According to the results of the study shown in Tables 2-4, the effects of different levels of Ca, P and vitamin D_3 on variants, including Ca, P and Mg contents of blood plasma, P and ashes of bone, percentage of

Table 2: Effect of dietary Ca, P and vitamin D_3 on Ca, P and Mg level of plasma and tibia

	Tibia			Plasma		
Parameters	Ash (%)	P (%)	Ca (%)	Mg (mL)	P (mL)	Ca(mL)
Main effect						
Ca , P						
10% lower	52.58	7.316	20.160	2.400	7.166	27.75
Control	48.16	7.033	18.530	2.360	7.400	28.15
10% higher	51.58	7.300	20.330	2.300	7.450	26.43
SEM	14.20	0.278	0.563	0.750	0.372	1.832
Vitamin D_3	-	-	-	-	-	-
Control	52.16	7.200	19.690	2.430	7.300	26.85
10% higher	49.22	7.230	20.100	2.270	7.340	28.03
SEM	1.16	0.310	0.460	0.610	0.304	1.490
Intraction effect						
Ca , P	-	-	-	-	-	-
Vitamin D_3	-	-	-	-	-	-
Control	-	-	20.766^{ab}	-	-	-
10(%) higher	-	-	19.566^{ab}	-	-	-
Control	-	-	18.400^{a}	-	-	-
10% lower	-	-	18.766^{ab}	-	-	-
Control	-	-	21.132^{a}	-	-	-
10% higher	-	-	19.533^{ab}	-	-	-
SEM	-	-	0.796	-	-	-
P						
Ca and P level	0.111	0.067	0.091	0.640	0.850	0.790
Vitamin D_3 level	0.116	0.743	0.236	0.098	0.980	0.580
Intraction	0.508	0.510	0.046	0.451	0.901	0.960^{a}

^{a,b}Means within column with no common superscript differ significantly

Table 3: Effect of dietary Ca, P and vitamin D_3 on egg shell quality

Mani effect	Crack (%)	Soft shell (%)	Broken (%)	Losses (%)
Ca, P				
0 lower	0.981	1.280	0.306	2.550
Control	0.918	1.650	0.540	3.450
10% highter	0.485	0.905	0.351	1.920
SEM	0.210	0.412	0.121	0.566
Vitamin D_3				
Control	0.767	1.608	0.430	2.970
10% higher	0.822	0.954	0.368	2.310
SEM	0.210	0.412	0.121	0.462
P				
Ca and P	0.202	0.410	0.479	0.204
Vitamine D_3	0.729	0.142	0.549	0.377
Interaction	0.828	0.607	0.694	0.282

^{a,b}Mean within column no common superscript

Table 4: Effect of dietary Ca, P and vitamin D_3 on performance FCR hatchability (%)

Factors	Egg weight (%)	Production (%)	FCR	Hatchbility (%)
Main effect				
Ca , P				
10% higher	63.888	84.695^{a}	3.24^{b}	94.361
Control	63.548	83.089^{ab}	3.33^{b}	91.839
10% Lower	64.175	81.172^{b}	3.54^{a}	93.420
SEM	0.642	0.502	0.197	0.878
Vitamin D_3				
Control	63.737	82.163^{b}	3.440^{a}	91.690^{b}
10% higher	64.003	83.808^{a}	3.304^{b}	94.720^{a}
SEM	0.524	0.410	0.207	0.717
Intraction effect				
Ca , P				
Vitamin D_3				
Control	64.500	79.063^{b}	3.580^{a}	92.184^{ab}
10% higher	63.840	83.280^{a}	3.510^{a}	94.670^{a}
Control	62.620	82.670^{a}	3.520^{a}	89.710^{a}
10% higher	64.470	83.500^{a}	3.140^{b}	93.960^{b}
Control	64.080	84.750^{a}	3.230^{b}	93.184^{b}
10% lower	63.690	84.630^{a}	3.250^{b}	95.538^{a}
SEM	0.908	0.711	0.297	1.240
P				
Ca and P level	0.791	0.001	0.050	0.700
Vitamin D_3 level	0.727	0.017	0.041	0.028
Intraction	0.349	0.028	0.046	0.047

^{a,b}Means Within column with no common superscript differ significantly

hairline fracture, soft and broken eggs, total losses and the egg weight were not significant. Considering (Table 2), the influences of Ca, P and vitamin D_3 on Ca contents of bone ashes were not noticeable whereas there was a significant interaction among them in this regard ($p<0.05$). Concentrations of Ca, P and Mg are shown in Table 2 and as it is seen, there was no difference among them.

For these, parameters were not influenced by hatching time, they could not be proper indicators for various levels of Ca and P which was in agreement with previous observation by Orban *et al.* (1992). No clear differences in quality of egg shell could be detected between different levels of Ca, P and vitamin D_3 (Table 3). Finding of other studies suggested that qualitative improvement of egg shell is related to the weight (Gerber, 2006). The effects of Ca, P and vitamin D_3 on the egg weight and production, dietary conversion coefficient and hatching are shown in (Table 4). As it is deduced from these results, the average of egg weight was not affected by experimental factors. According to assays carried out by Safaa *et al.* (2008) and Keshavarz (2003), the egg weight was influenced by dietary protein, choline, folic acid and vitamin B_{12} and different amounts of Ca, P and vitamin D_3 couldn't

significantly affect the egg weight which is in consistent with the findings (Table 4). Data shown in Table 4 revealed that the effects of different concentrations of Ca and P on production percent were significant ($p<0.05$) and increasing Ca and P caused a loss in egg production. By reduction of P and Ca concentrations, production percent was increased drastically ($p<0.01$).

Also it can be observed that vitamin D_3 increments resulted in higher production percent ($p<0.05$). It is clear from Table 4 that the interaction between Ca, P and vitamin D_3 levels was fully marked ($p<0.05$) and decreasing P and Ca, in combination with increasing vitamin D_3 increased production percent severely. However, it was already suggested that addition of Ca caused the egg production to increase (Oliveira *et al*., 2002; Pizzolante *et al*., 2009).

CONCULSION

It is observed that Ca and P concentrations of 10% lower than in control were appropriate levels and that the incorporation of higher values of Ca and P (i.e., 2.56 and 0.33%, respectively) suffered from an oversupply that limited the availability of other nutrients (NRC, 1994). The Table 4 also implies that increasing vitamin D_3 from 3000-3300 I.U. added up to an increase of 82.163-83.808% proving that addition of vitamin D_3 causes improvement of releasing Ca and P which is similar to the findings of Oliveira *et al*. (2002) and Pizzolante *et al*. (2009). It is shown in the Table 4 that the influences of Ca and P on hatching were not significant and this outcome was in contrast with those of Novo *et al*. (1997) who reported that the hatchability of fertile eggs was declined by decreasing P amounts and it might owe to lack of any P deficiency among the levels tested here. Furthermore, the researchers observed that an increase of 3000-3300 I.U. in concentration of vitamin D_3 increased the hatching rate from 91.69,94.72% ($p<0.05$).

The rationale behind such an increase could be the increase in Ca content of blood (Table 4), as has been noted earlier (Orban *et al*., 1992; Fritts and Waldroup, 2003). In the case of feed conversion coefficient, considerable effects of P and vitamin D_3 were again found (Table 4). Considering that there was a direct relationship between feed conversion coefficient and egg production and weight, one can said that the feed conversion coefficient is well-correlated with egg production an weight. From Table 4, it is obvious that Ca and P of 10% lower than in control have been optimal and reasonable choices, evidenced by diminished feed conversion coefficient after using higher amounts of Ca and P. Indeed, it is indicated that the increase of 3000-3300 I.U. in the amount of vitamin D_3 had a positive effect on practical variables (Table 4). The combined results suggest that high levels of Ca and P had no dramatic influence on egg shell quality or they were far more than required, resulting in blocking other dietary nutrients and making them out of reach and thus, leading to a diminution in feeding efficiency. In other words, consisting of standard levels, the control treatment was an overdose of Ca and P and by a 10% reduction in concentration of these nutrients, strong effects on performance variables could be observed. Additionally, increase of vitamin D_3 improved this effect drastically ($p<0.05$).

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