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Effects of Mediterranean Mussel Shell (*Mytilus galloprovincialis*) on Performance and Egg Quality in Laying Quails

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Abstract

The effect of Mediterranean mussel shell *(Mytilus galloprovincialis)* (MMS) on performance, egg quality and some blood parameters were studied on quails *(Coturnix coturnix Japonica)* for a period of 10 weeks (13-23 weeks). A total of 90 quails were randomly separated into one control and two treatment groups. Each group was divided into six subgroups, each containing 5 animals. MMS was replaced with lime stone at the ratios of 50 and 100% in first (50% MMS) and second (100% MMS) group rations respectively. The diets were prepared to be isocaloric and isonitrogenous. Rations and water were given *ad libitum*. Ca source replacement did not significantly affect body weight, egg weight, egg yield, feed intake and feed conversion ratio of laying quails. The effects of MMS replacement on shape index, yolk index, yolk color, blood Ca, Mg, P levels with Mg and P levels of egg shell had no significance. Ca levels of egg shell decreased ($p<0.05$) in treatment groups however, the amount of crude ash of tibia was not altered. In sum, dietary MMS did not alter egg quality of laying quails. It may be concluded that MMS can be replaced with limestone in the diet of laying quails.

Keywords: Laying quails, mussel shell, performance, egg quality

Introduction

Blue mussel (*Mytilus galloprovincialis*) is cultivated or collected for human consumption in most of the coastal regions of Turkey. The production amount of all mussel species in Turkey from 2000 to 2016 differed from 9000 to 59000 ton annually, and the amount of blue mussel reached 12000 ton per year from 78 ton per year during these years (Anonymous, 2018). The shell percentages of mussels collected from different parts of Spain were determined between the ratio of 52 and 61% (Fuentes et al., 2009). Nearly 4500 to 28000 ton mussel shell waste was produced in Turkey annually. Marin and Luquet (2004) reported that 1-5% of the mollusc shell weight included organic matrix (aragonite, calcite, or in particular cases, vaterite). Other parts of shell were composed by calcium carbonate (Barros et al., 2009).

There were a number of studies on dietary organic Ca sources on layers except limestone (Ahmed et al., 2013; Çetin and Gürcan, 2006; Houndonougbo et al., 2012; Kismiati et al., 2012; Scheideler, 1998). There were also several studies (Ayaşan and Okan, 1999; Cufadar, 2014; Ertaş et al., 2006) on different sources (egg shell, oyster shell, mussel shell) and different sizes of dietary Ca in quails. Dietary replacement (25, 50, 75 and 100%) of limestone with eggshell (Gongruttananun, 2011) and limestone with eggshell and/or oyster shell (Cufa-

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dar, 2014) and limestone with mussel shell (Ertaş et al., 2006) were studied on layers. Gongruttananun (2011) and Cufadar (2014) reported that dietary replacement (50 and 100%) of limestone with eggshell and dietary oyster shell did not affect egg weight and egg production of layers respectively. On the other hand Ertaş et al. (2006) reported that egg production of quails improved when the ratio of 75% of limestone in the diet was replaced with mussel shell under heat stress conditions. Besides these investigations, the effects of dietary particle sizes of Ca sources were studied on layers (Safaa et al., 2008; Tunç and Cuhadar, 2015); and it was declared that dietary large particle sized of egg shell and oyster shell (Tunç and Cuhadar, 2015) or dietary replacement of fine lime stone to coarse lime stone or oyster shell (Safaa et al., 2008) did not affect laying performance of layers. It was reported that normal levels of dietary Ca (3% for laying quails and 3.75% for laying hens) with lower levels of dietary P (0.4% for laying quails and 0.53% for laying hens) has positive effect on egg production of layers without concerning source of mineral feed (Ahmad and Balander, 2003; Ayaşan and Okan, 1999). As reported by Lichovnikova (2007), there were also positive effects on egg shell weight and quality due to *ad libitum* oyster shell consumption. Some of these dietary Ca investigations was worked on especially late periods of layers (Cufadar, 2014; Safaa et al., 2008).

There were also several studies on the use of aquatic Ca sources on layers (Bugdayci et al. 2016; Pelícia et al., 2007). Bugdayci et al. (2016) declared that the replacement of cuttlefish bone with limestone at the levels of 50 and 100% did not affect productive performance and quality of eggs in japan quails. Moreover, Pelícia et al. (2007) used a source of marine calcium from marine alga (*Lithothamnium* sp.) in the diets of layers and reported that marine calcium could be replaced up to 45% of limestone without any effect on production and egg quality.

Recently, mussel has been cultivated for human diet. Many investigations have been carried out by using different sources and sizes of Ca in layers; however, experiments involving Mediterranean mussel (*Mytilus galloprovincialis*) shell (MMS) as a Ca source are lacking in literature. The availability of Ca content of MMS may be altered by salty water. This may have a positive effect on performance and egg quality for layers. There is a study which is carried out with fresh water mussel shell (*Unio elangatus eucirrus Bourguignat*) at the same dietary proportions as our study in quails. However, there is no study on the use of 50% and 100% MMS in laying quails. The aim of this investigation is to determine the possible effects of Mediterranean mussel shell on productive performance, internal – external egg quality and some blood parameters of japan quails.

Materials and Methods

Animals, management and rations

Protocols of animal use, animal care and research protocols were approved (2015-121) by local ethical committee of Burdur Mehmet Akif Ersoy University as required. 13 week-old 90 quail (*Coturnix coturnix japonica*) were used in the investigation. The quails were randomly allocated into one control and two treatment groups. Each group contained 30 quails. All groups were divided into six replicates (each contained 5 quails). Quails were kept in cages (45cmx50cmx22cm) in a windowed house. 8/16 h dark/light regimens were applied in the windowed house. Ration and water were given *ad libitum* during the 10 weeks of experiment. The ingredients and chemical composition of all groups' diet are presented in Table 1. The diets consisted of two levels of MMS (50 and 100%) by limestone in the diets of first and second treatment groups, respectively.

Basal diet's nutrient composition was determined by using AOAC (1990) directives. Titus and Fritz (1971) equation was used to estimate metabolizable energy of diets.

The $CaCO_3$ content of MMS was determined in the laboratory. For this purpose, weighed amounts of MMS were burned in an ash furnace, crumbed and waited in 37% HCl for 12 h. After the waiting period, the mixture of MMS and HCl was filtered from Whatmann paper. Paper was dried (105°C) in a stove (Memmert UE500, C593.0011, Germany) and burned (550°C) in ash furnace (Carbolite, S302RR, UK). The Ca level of MMS (38.5%) was determined by using the amounts of acid soluble ash (96.19%) of MMS and total Ca levels of group rations were illustrated in Table 1.

Traits recorded and methods applied

Animals were weighed individually at the beginning and at the end of the trial. Quails were weighted by using a precision balance (Model: HGM-20K-ER8412, 1g sensitivity, UWE CO, Taipei-Taiwan) and mortality was recorded as it occurred.

Eggs were gathered daily and egg production was stated on a hen-day basis. Laid eggs throughout the last two consecutive days of every week were weighed individually. For this purpose, precision balance (Model CP224S-14105100, 0.1 mg sensitivity, Sartorius AG, Göttingen-Germany) were used for determine the egg weight. Feed consumption was recorded weekly and calculated (g/day/quail). The feed efficiency was calculated in two type (g feed/g egg and g feed/dozen egg).

Egg quality

Internal egg quality were determined by the fallowing procedure. 12 eggs laid at 09.00 - 12.00 h were collected randomly from each group in total (2 eggs from each replicate) on

Table 1. Ingredients and chemical composition of control and treatment groups' diet

	Treatment groups		
Ingredients of diets, (%)	**Control**	**50% MMS**	**100% MMS**
Corn	45.55	45.55	45.55
Wheat	7	7	7
Soybean meal (48%)	18	18	18
Full fat soy	10	10	10
Sunflower meal (36%)	9	9	9
Vegetable oil	2.8	2.8	2.8
DCP	1.5	1.5	1.5
Limestone	5.4	2.7	-
Mediterranean mussel shell	-	2.7	5.4
Salt	0.3	0.3	0.3
Vit-Min complex*	0.25	0.25	0.25
DL-Methionine	0.1	0.1	0.1
L-Lysine	0.1	0.1	0.1
Analysed chemical composition of diets as feed, (g/kg)			
Crude protein	174.9	171.4	174.6
Eter extract	80.0	79.0	79.3
Crude fibre	40.7	41.4	40.9
Crude ash	100.1	98.8	102.8
Dry matter	918.4	918.2	919.1
Ca**	25.00	25.15	25.30
ME (kcal/kg)	2561.76	2529.37	2524.60

*Each kilogram of vitamin-mineral mix contains 12 000 000 IU A vit, 20 000 mg E vit, 50 000 mg Mn, 50 000 mg Fe, 50 000 mg Zn, 10 000 mg Cu, 800 mg L, 150 mg Co, 150 mg Se;
** Calculated levels

4th, 6th, 8th and 10th week of the experiment (as a total of 48 eggs per group throughout the experiment). Each egg was weighed with a precision balance individually and shape index of eggs were calculated (Shape index=(egg width/egg length)x100).

The egg content was broken onto a glass plate. Then, the height of the albumen and the yolk was measured with a tripod micrometer (Mitutoyo, No: 2050S-19, 0.01-20 mm; Kawasaki-Japan). Albumen length, albumen width and yolk diameter were measured with digital calliper.

Yolk index [(yolk height/yolk diameter) x 100], albumen index [(albumen height/average of albumen length and albumen width) x 100] and Haugh units [[100 x log (H+7.57-1.7W0.37)] where H is albumen height and W is egg weight] were calculated by using the values of albumen length, albumen width and yolk diameter (Card and Nesheim, 1972). Roche yolk color fan was used for scoring egg yolk colors of the treated eggs on a glass plate (Vuilleumier, 1969).

Internal and external quality analyses of egg were completed within the next 24 h after the eggs were gathered. Egg weights of treated eggs were used to evaluate of their quality individually.

Ca, P and Mg content of egg shells of control and treatment groups were detected by ICP-OES (Perkin Elmer ICPOES Optima 8000, USA). For this purpose, all treated egg shells were collected and mixed in a poll of their own subgroups. In sample preparation 0.3 g of shell ash and 6 ml HNO_3 (65%) treated in microwave (Milestone Stard D) according to the method of Al-Obaidi et al. (2012).

Serum analysis
At the end of the experiment, two quails from each replicate (12 from each group) were randomly selected and slaughtered. Blood samples were gathered at the slaughtering operation and centrifuged at 3000xg for 10 min. individually. Fresh serum samples were analyzed to determine of serum Ca, P and Mg levels by autoanalyser (Model: Gesan-Chem200, No:

Table 2. The effects of dietary treatments on initial and final body weights of laying quails (g)

	Dietary treatments			
	Control	50% MMS	100% MMS	p
Initial body weight (g)	274.93±4.11	270.26±5.44	270.03±4.45	0.709
Final body weight (g)	277.68±5.12*	283.48±6.64*	284.76±6.10	0.675

Control: 100% limestone; T1: 50% MMS + 50% limestone; T2: 100% MMS; n=30; *n=29; p<0.05

Table 3. The effects of dietary treatments on performance of laying quails (mean±SE)

	Dietary treatments			
	Control	50% MMS	100% MMS	p
Feed intake (g/day per quail)	34.68±1.12	33.29±1.25	33.54±0.64	0.615
Egg production (%)	92.06±3.68	93.46±2.92	93.52±3.64	0.944
Egg weight (g)	13.38±0.10	13.07±0.28	13.23±0.13	0.522
FCR* (kg feed / kg egg)	2.59±0.09	2.54±0.07	2.53±0.03	0.829
FCR (kg feed / dozen egg)	0.43±0.02	0.42±0.02	0.40±0.02	0.764

Control: %100 limestone; T1: 50% MMS + 50% limestone; T2: 100% MMS; FCR: Feed conversion ratio; n=6 per group.

Table 4. The effects of dietary treatments on egg traits of laying quails (mean ±SE)

	Dietary treatments			
	Control	50% MMS	100% MMS	p
Weight of treated eggs (g)	13.42±0.16^{a}	12.91±0.31^{b}	13.26±0.06^{a}	0.011
Crude ash of tibia (%)	64.14±1.38	63.55±1.56	61.81±2.69	0.688
Shape index (%)	77.32±0.41	77.29±0.44	76.31±0.51	0.223
Albumen high (mm)	5.08±0.12^{a}	4.54±0.40^{b}	4.87±0.42^{ab}	0.013
Albumen index (%)	11.77±0.33	10.53±0.27	11.07±42	0.057
Yolk index (%)	42.37±0.37	41.65±0.35	41.32±0.25	0.085
Haugh unit	91.10±0.67^{a}	88.61±0.65^{b}	90.36±0.64^{ab}	0.033
Egg yolk color[1]	10.08±0.12	10.07±0.12	10.08±0.13	0.996

Control: %100 limestone; T1: 50% MMS+50% limestone; T2: 100% MMS
^{a,b} Means within a row followed by the different superscripts differ significantly (p<0.05)
[1]Roche color scores are based upon Roche Color Fan Edition 1965, n=12

1102422, Campobello-Italy) using its commercial kit (Monoreagent-LR-C2230150V, Italy).

Determination of tibia ash

The right legs of slaughtered quails (12 from each group) were removed and placed in an autoclave for the separation of meat and bone. Separated tibias were dried at 105°C for 12 h in an incubator. Then, tibias were burned at 550°C for 5 h in an ash furnace.

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) program (SPSS Inc., Chicago, USA) used for statistical analyses. Kolmogorov-Smirnov test used for checking the normality of the data. The differences among groups examined (One-way ANOVA). Duncan was used for the significance of mean differences between groups. Quality characteristics of egg were analyzed after detecting egg weight. Egg internal and external quality characteristics were analyzed after adjusting egg weight and values were given as conjectural marginal means and standard error of mean. p<0.05 was determined for the level of significance (Dawson and Trapp, 2001).

Results

Dietary MMS did not alter the final body weight (Table 2), productive performance, feed intake, egg weight and feed

Table 5. The effects of dietary treatments on Ca, Mg and P of egg shell ash (%) and Ca, Mg, P levels of blood serum (mg/dL) of laying quails, (mean±SE)*

	Dietary treatments			
	Control	50% MMS	100% MMS	p
Egg shell (%)				
Ca	41.77±2.01[a]	21.37±5.67[b]	19.73±4.53[b]	0.004
Mg	0.63±0.02	0.61±0.04	0.71±0.02	0.098
P	0.25±0.03	0.28±0.02	0.30±0.01	0.332
Blood serum (mg/dL)				
Ca	21.78±2.64	24.96±1.50	24.87±2.04	0.496
Mg	2.65±0.05	2.65±0.03	2.56±0.03	0.276
P	34.10±1.62	36.84±1.71	35.05±1.04	0.440

Control: %100 limestone; T1: 50% MMS + 50% limestone; T2: 100% MMS; n=6; $p<0.05$

conversion ratio of quails (Table 3) in the present study. Egg quality parameters results, such as shape index, albumen index, yolk index and egg yolk color were not altered by dietary MMS. However, albumen high and Haugh unit of treated eggs differed (Table 4). Calcium levels of egg shell were significantly decreased ($p<0.05$) by the replacement of lime stone with mussel shell in both ratios (Table 5). On the other hand, Mg and P levels of egg shell were not affected in the present study. Crude ash of tibia was not changed by dietary limestone replacement (Table 4). Dietary substitution of limestone with mussel shell did not alter Ca, Mg and P levels of blood (Table 5).

Discussion

In the present study, egg production of quails was not affected by the replacement of limestone with MMS. This result was similar to lime stone replacement with cuttlefish bone in laying quails (Bugdayci et al., 2016) and also similar with oyster shell and egg shell in laying hens (Cufadar, 2014). Beside this, Scheideler (1998) declared that the replacement of 25 of limestone with oyster shell did not affect egg production in laying hens. On the other hand, Ertaş et al. (2006) reported that the replacement of 75% of limestone with fresh water mussel shell in quail rations increased egg production. Hence, we can state that, the availability of fresh water mussel shells or salty water mussel shells as a Ca source may be different for quails.

There are several investigations which reported that the effects of different Ca sources and different sizes did not change the egg production of layers (MacIsaac et al., 2016; Safaa et al., 2008; Tunç and Cuhadar, 2015). However, Pelicia et al. (2011) declared that increasing Ca levels (3 to 4.5%) of laying hen rations linearly decreased productive performance of laying hens. On the other hand, Pizzolante et al. (2006) reported that different dietary Ca levels (3.5 and 4.0) did not alter the productive performance of semi-heavy layers without concerning limestone particle composition. Studies also showed that laying performance of layers is related with the dietary ratio of Ca/P; dietary Mg and P levels as well as size with/or source of dietary Ca (Ahmad and Balander, 2003; Ayaşan and Okan, 1999; Skřivan et al., 2016).

The results of this study regarding feed intake and egg weight of quails agree with the report of Ertaş et al. (2006) who did not find any difference on feed intake and egg weight when 25, 50, 75 and 100% of fine limestone in the diet of laying quails was substituted with mussel shell. Similarly, Bugdayci et al. (2016) reported that replacement of limestone with the ratio of 50 and 100% of cuttlefish bone did not significantly affect the feed intake and egg weight of laying quails. On the other hand, the replacement (50%) of limestone with egg shell, the replacement (50%) of limestone with oyster shell (Cufadar, 2014), and the replacement (100%) of limestone with whelk shell in laying hen rations (MacIsaac et al., 2016) decreased feed intake without affecting egg weight. There are also several studies (Cufadar, 2014; MacIsaac et al., 2016) reported that alternative Ca sources could decrease feed intake of layers.

Cufadar (2014) reported that the replacement of the ratio of 50 and 100% proportions of oyster shell and egg shell did not alter the feed conversion ratio of laying hens, which is consistent with the relating result of this study. In addition to this report, MacIsaac et al. (2016) declared that the use of grounded whelk shell (100%) in the rations at high temperature conditions decreased feed conversion ratio of laying hens. The present study was completed during normal environmental temperature.

Quality parameters of treated eggs except albumen high and Haugh unit were not altered by the substitution of lime stone with mussel shell; on the contrary, Wang et al. (2014) reported that large dietary particle Ca sources (oyster shell and limestone) increased albumen height and Haugh unit in layers. The replacement of limestone with mussel shell at the ratio of 50% had lower albumen high and Haugh unit when compared with control group ($p<0.05$). This result might be caused by the weights of treated group eggs which selected randomly (Table 4). The use of large particle Ca sources (oyster shell and limestone) in layer diets did not affect yolk color of eggs mentioned by Wang et al. (2014), which is similar with the present study.

Dietary MMS decreased the Ca levels of egg shell in the present study; however, Bugdayci et al. (2016) reported that dietary cuttlefish bone did not alter the Ca levels of egg shell in quails. This result may be caused by the difference of the Ca availability of dietary MMS and cuttlefish bone. Also, Wang et al. (2014) reported that large dietary particle Ca sources (oyster shell and limestone) increased shell content of phosphorus and magnesium when compared with small particle sizes. On the other hand, Skřivan et al. (2016) declared that dietary limestone size (fine or ground) did not affect shell percentages (weight of egg shell / weight of egg) of laying hens. Besides these results, Wang et al. (2014) reported that dietary oyster shell instead of limestone in laying ducks caused lower Ca accumulation in egg shell. It is clear that as an aquatic additive, mussel shell like cuttlefish bone (Bugdayci et al., 2016) or chitosan (Świątkiewicz et al., 2018) had different effects on bio-mineralization of organism. Bugdayci et al. (2016) reported that dietary replacement of Ca source with an aquatic one did not change the amount of crude ash of egg shell and tibia in laying quails. However, Świątkiewicz et al. (2018) declared that dietary chitosan supplementation increased egg shell thickness of laying hens at late production period. There were several studies declaring the egg shell mineral content alteration fed with different dietary Ca sources and different size of these sources (de Witt et al., 2009; Tunç and Cuhadar, 2015).

The amount of crude ash of tibia was not affected in the study; and this result was similar with the results regarding the use of egg shell as a Ca source and the use of different amounts (3.5-4.7%) of Ca in laying hen in Gongruttananun (2011) and An et al. (2016) respectively. Wang et al. (2014) also declared that the use of dietary small particle sized Ca source in laying ducks did not affect the ash content of tibia in laying ducks. Similarly, Pelicia et al. (2011) reported that increasing Ca levels of laying hen rations from 3.0 to 4.5% did not alter tibia ash of layers at the age of 35 weeks old. It is clear that the size or the source or the dietary amount (not less than 3.5%) of Ca additives did not effect on total mineral content of tibia for laying ducks (Wang et al., 2014) and hens (An et al., 2016; Gongruttananun, 2011). However, Tunç and Cuhadar (2015) reported that the levels of Ca, P and Mg of the tibia changed with different sources and proportions of dietary Ca in laying hens.

Dietary substitution of limestone with MMS decreased the levels of egg shell Ca content; however, blood Ca levels were not affected as well as blood levels of Mg and P. This result may be caused by the source of available Ca. On the other hand, Pelicia et al. (2011) reported that the blood Ca levels of laying hens were affected by the dietary Ca levels of layers. In the present study, the levels of analyzed dietary Ca in control and treatment groups were similar. For this reason, blood Ca levels of quails may not have been altered. Besides these, the source and the proportions of Ca did not affect egg production and crude ash of tibia in the present study.

Bugdayci et al., (2016) and Gongruttananun, (2011) reported that 50% and 100% dietary limestone replacement with eggshell or cuttlefish bone did not alter the blood Ca levels of laying quails and laying hens respectively. These reports were similar to the results of the present study. However, the levels of calcium in the blood and eggshell were not strictly correlated to each other. This may be caused by the availability of different Ca sources.

Related with the production of Mediterranean mussel, the amounts of mussel shell by-product can reach to 28000 tons annually. This amount of mussel shell could be brought into the economy. If the MMS is considered as a source of calcium in animal feeding, the environmental pollution also will decrease. In the present study the results point out that total substitution of limestone with MSS in the diet does not have negative effect on the laying performance of quails or quality parameters of eggs.

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