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The effects of rations containing *ad libitum* limestone grit with whole flaxseed on quails' performance, egg quality, and blood parameters

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Abstract: The purpose of this research is to determine the effects of consuming feed rations containing *ad libitum* limestone grit with whole or ground flaxseed (10%) on quails' performance, egg quality (shape index, albumen index, yolk index, and Haugh unit), and some blood parameters (hematocrit, cholesterol, and triglyceride) during a period of 8 weeks. Five weeks old quails (*Coturnix coturnix japonica*; 140 in total) were divided into control and 4 trial groups. The rations of the first (W) and second (G) trial groups consisted of whole or ground flaxseeds (10%), respectively. The composition of the rations of the third (Wg) and fourth (Gg) experimental groups was similar to that of the W and G groups. However, these groups (Wg and Gg) were allowed to consume additional *ad libitum* grit. The *ad libitum* grit consumed by the Wg and Gg groups did not affect their egg production. Except for the yolk index, all other parameters of egg quality were not affected by ration manipulations in the trial. A higher amount of grit was consumed by Wg group; however, it did not cause a statistically significant difference in them compared to the Gg group in the trial. It is discovered that the group that consumed whole (10%) or ground (10%) flax seeds has an increased yolk index compared to those groups that consumed *ad libitum* grit.

Key words: Egg quality, flaxseed, limestone grit, performance, quail

1. Introduction

Flaxseed (*Linum usitatissimum*) with high omega-3 fatty acid, alpha linoleic acid, lignans, and fiber content is a good source of feed enhancer [1]. Poultry feed rations containing flaxseed rich in omega-3 fatty acids are used because the fatty acids can be transferred to egg yolk [2]. Owing to consumers' demands for foods rich in omega-3 fatty acids, researchers [3–5] became interested in modifying the fatty acid profile of eggs by using flaxseeds. Flaxseeds with high fiber content have 20% solubility [6]. In addition, the lignans of flaxseed are found to be equally or somewhat more potent than Butylated Hydroxytoluene (BHT) and Vitamin E. Thus, they can have commercial potential as an alternative to these antioxidants [7]. However, the high omega-3 fatty acid content of flaxseeds has a negative effect on the oxidative status of egg yolk [2]. The grounded form of flaxseeds is preferred in animal rations used for commercial purposes. On the other hand, its ability to oxidize lipids quickly is another risk as it deteriorates feeds rapidly. In this context, the grinding function of chickens' gizzards becomes prominent.

One of the important functions of grit is that it assists chicken gizzard in enhancing the mechanical processing

of feeds; and the shape of grit in the gizzard affects its muscularity [8]. Insoluble flint stone and granite help to evaluate the maximum level of feed consumption. There is also soluble limestone or marble in grit containing calcium. Poultry breeders in Turkey generally use these cheap and plentiful sources of grit. Grit containing granite stone increases the capacity of gizzard up to 50% [9]. Bale-Therik et al. [10] stated that grit could help to improve the performance of local chickens as well as their daily feed consumption, daily body weight gains, and feeding efficiency.

Recent investigations show that the consumption of whole cereals diet by poultry had no beneficial effects [11] and its higher consumption had negative effects [12]. On the other hand, Eser et al. [13] reported that broilers' rations containing grit with maize and barley increased the relative weight of the broilers' gizzard and decreased their abdominal fat weight. Garipoğlu et al. [14] stated that the voluntary intake of insoluble granite grit by broilers did not affect their growth performance.

The current research aims to evaluate the effects of the consumption of rations containing *ad libitum* limestone grit with whole and ground flaxseed on quails'

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laying performance, egg quality, and blood parameters (hematocrit, cholesterol, and triglyceride).

2. Materials and methods

The research on animals was conducted according to the Ethics Committee of Burdur Mehmet Akif Ersoy University on animal use (93773921-14). It was carried out in Burdur, Turkey with coordinates of 37°41'33.9"N and 30°20'54.4"E.

2.1. Animals and diets

One hundred and forty quails (*Coturnix coturnix japonica*; 5 weeks old) were used in the experiment. Control and four experimental groups, each consisting of four subgroups (7 quails each), were formed in the study. Quails were provided with light 16 h per day and housed in a commercial quail cage (45cm × 50cm × 22cm). The research continued for 8 weeks. Rations were formulated according to NRC [15] (Table 1). Feed and water were supplied *ad libitum*. The rations of the control group did not contain flaxseeds. The first (W) and second (G) trial groups' rations were composed of whole or ground flaxseeds (10%), respectively. The third and fourth trial groups' (Wg and Gg) rations were similar to those of the first and second groups, respectively, the only difference being that the third and fourth groups were given grit *ad libitum* by using different feedbox. The average diameter

of limestone (marble) grit given *ad libitum* was calculated as 2.071 mm.

The nutritional compositions of the flaxseeds and the rations of the control and trial groups were determined according to AOAC [16]. All the groups' rations were analyzed for crude fiber according to Crampton and Mynard [17]. The fatty acid composition of the flaxseed was detected with GC-MS (Agilent 5975 C Agilent 7890A GC) using the method of Bligh and Dyer [18].

2.2. Performance parameters

The quails were weighed twice, at the beginning and end of the trial. The eggs of each subgroup were recorded and collected daily. The numbers of eggs were presented in hen-day basis. Weekly egg weights of the groups were determined by collecting all eggs at the end of the week and weighing them individually on the next day. Weekly feed consumption for all groups was calculated as daily feed consumption per quail. Feed efficiency was expressed as kg feed consumed per kg egg, and kg feed consumed per dozen eggs. Dead quails were recorded daily during the experiment.

2.3. Egg quality criteria

The forty eggs from each group (2 eggs from each subgroup) were collected biweekly for the measurement of internal egg quality criteria. Each egg was weighed; and their shape indexes were calculated [(egg width/egg

Table 1. Ingredients and chemical composition of control group and trial groups diets.

Ingredients (%)			Chemical composition (analyzed)		
	Control group	Treatment groups		Control group	Treatment groups
Corn	44.45	40.05	Crude protein (%)	19.56	19.21
Wheat	7.00	7.00	Ether extract (%)	8.42	8.30
Soybean meal	18.00	18.00	Crude fibre (%)	4.64	4.83
Full fat soybean	10.00	5.50	Crude ash (%)	11.99	10.72
Sunflower meal	9.00	9.00	Dry matter (%)	91.80	94.57
Canola oil	3.00	1.90	ME ^b (kcal/kg)	2901	2904
Dicalcium Phosphate	1.40	1.40			
Flaxseed	-	10.00			
Limestone	6.40	6.40			
Salt	0.30	0.30			
Vit.-Min. ^a	0.25	0.25			
DL-methionine	0.10	0.10			
L-lysine	0.10	0.10			

^a Each kg 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 I, 150 mg Co, 150 mg Se.

^b Calculated levels of metabolizable energy (ME).

length) \times 100]. The albumen and yolk heights of the eggs broken on the glass table were measured with the help of a tripod micrometer (Mitutoyo, No. 2050S-19, 0.01-20 mm; Kawasaki, Japan). A digital caliper was used for other measurements (length and width of albumen, diameter of yolk) of eggs. Yolk index (Y), albumen index (A), and Haugh unit (H) of eggs were calculated by using the following formulas [19]: $Y = (\text{yolk height/yolk diameter}) \times 100$; $A = (\text{albumen height/average of albumen length and albumen width}) \times 100$; $H = 100 \times \log(\text{albumen height} + 7.57 - 1.7 \times \text{egg weight} \times 0.37)$.

All egg quality analyses were completed for individually weighed eggs within 24 h after their collection. At the end of the trial, an egg yolk pool was created [20] for each group by selecting one egg from each subgroup to determine the egg yolk cholesterol and the egg yolk fatty acids by combining each replicate of egg yolk.

2.4. Determination of egg yolk cholesterol

For the cholesterol analysis, egg yolk samples were blended with 10 mL ethanolic KOH and 0.3 g pyrogallol. They were saponified at 80 °C for 15 min. Water (5 mL) and 2 \times 20 mL of hexane were added. The organic phase was evaporated in a water bath. The residue dissolved in 1 mL of mobile phase was introduced into the HPLC system (Shimadzu Prominence HPLC). It consisted of the followings: CBM: 20ACBM; detector: DAD (SPD - M20A); column part: CTO - 10ASVp; pump: LC20 AT; autosampler: SIL 20ACHT; computer program: LC Solution. The mobile phase was modified by using 45% acetonitrile, 45% isopropyl alcohol, and 10% water [21]. It consisted of the followings: Column: Kromasil 100 5 C-18 (250 \times 4.6 mm, 5 μ m); flow rate: 1.5 mL/min; column temperature: 40 °C; injection volume: 50 μ L. Results were determined in 210 nm.

2.5. Determination of fatty acids

In order to determine the fatty acids of egg yolks and flaxseed, 20 mL chloroform: methanol (2:1) was added to the egg yolk samples and grounded flaxseed. Organic phases were evaporated after the extraction. Extracted oils were derivatized with 1.5 M methanolic HCL and injected into the GC-MS system (Aligent 5975C Aligent 7890A GC) equipped with a detector and column (HP-88) (100 \times 0.25 \times 0.20 μ m). The column temperature was programmed at 60 °C/min from 175 to 215 °C. The temperature of the injector and detector was set to 250 °C [18].

2.6. Blood analysis

At the end of the trial, blood samples were collected via decapitation process from all groups (two samples from each subgroup); and blood serum was separated by centrifuge (3000 \times g; 10 min.). Fresh serum samples were treated by Autoanalyser mod Gesanchem 200 (Campobello, Italy; serial number: 1102422) to determine the cholesterol and triglyceride levels using their

accompanying commercial kits (Cholesterol monoreagent LR, ref C2230150V; Triglycerides monoreagent LR ref C4730150V respectively). Hematocrit was performed on the collected blood samples to anticoagulated tubes via decapitation process by using microhematocrit centrifuge (18,000 \times g; 5 min).

2.7. Statistical analyses

Statistical analyses were done using SPSS software (SPSS Inc., Chicago, IL, USA). The normality of data distribution was checked using the Kolmogorov-Smirnov test. One-way ANOVA was performed to examine the differences among the groups. The significance of mean differences between groups was tested by Duncan. The internal and external characteristics of eggs were analyzed after adjusting their weight, and values were given as estimated marginal means and standard error of mean. Level of significance was taken as $p < 0.05$ [22]. The model used to analyze the treated quails' parameters was:

$$Y_i = \mu + a_i + e_i$$

Where, Y_i : is the treatment, μ : is the overall mean, a_i : is the effect of dietary treatments, e_i : is the random residual.

3. Results

The fatty acid compositions of the egg yolk of all groups and flaxseed were analyzed (Table 2). Live weight, egg weight, egg production, feed consumption, and feed efficiency of the groups were not affected by the diet containing whole or ground flaxseed with/without *ad libitum* grit (Table 3).

During the experiment, four quails died: two from Wg group, one from W and another one from C group. Shape index, albumen index, yolk index, and Haugh unit data of the trial groups were not found significant compared with the control group, except yolk index (Table 4). Yolk index increased in all treatment groups ($p < 0.05$). Yolk index was not affected by the consumption of *ad libitum* grit. The serum levels of cholesterol and triglyceride were not altered by the feeding of *ad libitum* grit in the trial groups. The blood hematocrit values of all trial groups were not affected compared with the control group (Table 5). The average values of egg yolk cholesterol were 5.84, 7.45, 5.24, 5.77, and 7.70 mg/g egg yolk fat in the control and treatment groups, respectively. The mean values of limestone grit consumed by Wg and Gg groups were 0.52 g and 0.34 g per quail per day, respectively. Consumption of whole flaxseed numerically increased the *ad libitum* consumption of limestone grit. However, this difference was not significant (Table 6).

4. Discussion

Dietary supplementation of flaxseed did not affect live weight, egg weight, egg production, feed consumption, and feed efficiency of laying quails. Sepehr et al. [23] reported that a diet containing 9% whole or milled flaxseed did not

Table 2. Egg yolk cholesterol (mg/g egg yolk fat) and fatty acid composition of egg yolk with flaxseed (%).

	Flaxseed	Treatment Groups				
		C	W	G	Wg	Gg
Cholesterol	-	5.84	7.45	5.77	5.24	7.70
Fatty acid composition						
Myristic acid	0.186	0.44	0.32	0.44	0.42	0.46
Myristoleic acid	-	0.10	0.04	0.06	0.04	0.06
Pentadecanoic acid	-	0.07	0.01	0.01	0.06	0.04
Palmitic acid	8.1	26.29	26.05	23.88	23.01	22.78
Palmitoleic acid	-	4.49	2.08	3.49	3.51	5.86
Heptadecanoic acid	-	0.24	0.12	0.12	0.20	0.62
cis10-heptedecanoic acid	-	0.06	0.05	0.12	1.13	0.33
Stearic acid	6.12	7.91	15.56	8.10	11.54	7.32
Oleic acid	19.8	38.55	31.95	40.80	39.76	42.23
Linoleic acid	13.8	15.41	15.97	13.79	13.82	13.36
Arachidic acid	0.01	0.31	0.30	0.71	0.25	0.26
g-linolenic acid	47	0.62	1.00	5.28	4.24	5.37
cis-11,14 eicosadienoic acid	1.19	-	-	-	-	-
Heneicosanoic acid	0.01	0.10	-	-	-	-
11,14,17-eicosatrienoic acid	0.01	1.12	3.35	0.23	0.96	0.22
Nervonic acid	0.11	1.00	0.01	0.01	0.01	0.01
EPA	0.96	0.01	0.05	0.05	0.05	0.06
Tricosanoic acid	1.01	-	-	-	-	-
Lignoceric acid	0.01	-	0.08	0.38	0.04	0.05

C, control group; W, whole flaxseed consumed group; G, ground flaxseed consumed group; Wg, whole flaxseed + *ad libitum* limestone grit consumed group; Gg, ground flaxseed + *ad libitum* limestone grit consumed group.

Table 3. Performance parameters of laying quails.

Performance parameters	Dietary treatments					P
	C	W	G	Wg	Gg	
Initial body weight (g)	219.17 ± 6.13	224.64 ± 3.81	224.07 ± 3.99	218.96 ± 5.13	217.39 ± 4.77	0.766
Final body weight (g)	273.25 ± 5.35	265.50 ± 3.77	266.74 ± 5.03	265.65 ± 5.25	273.25 ± 5.18	0.618
Feed intake (g/day per quail)	34.30 ± 0.96	37.34 ± 0.86	36.29 ± 2.01	36.41 ± 1.08	35.63 ± 0.57	0.503
Egg production (%)	75.55 ± 3.52	80.16 ± 4.57	74.38 ± 3.73	74.05 ± 2.97	78.74 ± 3.14	0.693
Egg weight (g)	12.74 ± 0.15	13.13 ± 0.20	12.97 ± 0.13	12.54 ± 0.09	12.84 ± 0.17	0.139
Feed efficiency (kg feed per kg egg)	2.69 ± 0.08	2.84 ± 0.10	2.80 ± 0.16	2.90 ± 0.08	2.77 ± 0.02	0.690
Feed efficiency (kg feed per dozen egg)	0.54 ± 0.02	0.55 ± 0.02	0.58 ± 0.03	0.57 ± 0.01	0.54 ± 0.02	0.815

C, control group; W, whole flaxseed consumed group; G, ground flaxseed consumed group; Wg, whole flaxseed + *ad libitum* limestone grit consumed group; Gg, ground flaxseed + *ad libitum* limestone grit consumed group.

Table 4. Egg quality parameters of laying quails, (%).

Egg quality parameters	Dietary treatments					P
	C	W	G	Wg	Gg	
Shape index	78.01 ± 0.70	78.40 ± 0.36	77.18 ± 0.41	78.45 ± 0.37	78.32 ± 0.64	0.411
Albumen index	10.95 ± 0.37	11.02 ± 0.44	10.78 ± 0.38	10.68 ± 0.30	10.41 ± 0.23	0.764
Yolk index	44.88 ± 0.39 ^b	47.05 ± 0.39 ^a	47.10 ± 0.61 ^a	46.47 ± 0.56 ^a	47.06 ± 0.23 ^a	0.007
Haugh unit	93.73 ± 0.67	93.94 ± 0.84	93.29 ± 0.71	93.41 ± 0.55	92.65 ± 0.41	0.689

C, control group; W, whole flaxseed consumed group; G, ground flaxseed consumed group; Wg, whole flaxseed + *ad libitum* limestone grit consumed group; Gg, ground flaxseed + *ad libitum* limestone grit consumed group.

^{a,b} Means within a row followed by the different superscripts differ significantly (p < 0.05).

Table 5. Blood parameters of laying quails.

Blood parameters	Dietary treatments					p
	C	W	G	Wg	Gg	
Hematocrit (%)	44.37 ± 1.38	42.00 ± 1.50	41.25 ± 1.75	45.37 ± 1.25	42.37 ± 1.01	0.218
Cholesterol (mg/dl)	161.87 ± 9.16	157.12 ± 12.99	169.12 ± 14.78	147.50 ± 2.59	162.62 ± 14.57	0.760
Triglycerides (mg/dl)	1304.25 ± 164.04	1218.25 ± 234.38	1213.75 ± 301.68	986.87 ± 179.66	1152.62 ± 241.82	0.139

C, control group; W, whole flaxseed consumed group; G, ground flaxseed consumed group; Wg, whole flaxseed + *ad libitum* limestone grit consumed group; Gg, ground flaxseed + *ad libitum* limestone grit consumed group.

Table 6. *Ad libitum* grit consumption of quails (g/day per quail).

	Wg	Gg	p
Average grit consumption (g/day per quail)	0.52 ± 0.08	0.34 ± 0.05	0.141

Wg, whole flaxseed + *ad libitum* limestone grit consumed group; Gg, ground flaxseed + *ad libitum* limestone grit consumed group.

affect egg production and egg weight of commercial laying hens, which complies with the current trial. Another study, Bean et al. [3] reported that a diet containing 10% of flaxseed in laying hens' rations did not affect their feed consumption, egg weight and body weights. Squires et al. [24] showed that a diet containing 10% and 20% flaxseed decreased the body weight of laying hens, while a diet containing 20% flaxseed increased their feed consumption. An experiment tested the effects of ground or whole flaxseed diet of 5%, 10%, or 15%; it was reported that a diet containing 5% whole flaxseed significantly decreased body weight and feed consumption of laying hens, while a diet containing 10% ground flaxseed significantly increased their feed consumption [25]. This result was supported by Sepehr et al. [23] who found that a diet containing 9% milled flaxseed increased feed consumption levels of commercial layers. In addition

to these results, Halle and Schöne [26] and Antruejo et al. [27] reported that a diet containing 15% flaxseed cake and flaxseed reduced feed consumption in laying hens, respectively. On the other hand, Basmacıoğlu et al. [5] declared that diet containing 4.32% and 8.64% flaxseed did not affect feed consumption, feed efficiency, and egg weight of laying hens. However, while a diet containing 4.32% flaxseed increased egg production, a diet containing 8.64% flaxseed did not. Similarly, research on quails revealed that diet containing 2%, 4%, and 6% flaxseed significantly increased egg weight at all levels [28]. The differences may have occurred due to the nutritional ingredients of the rations, the physical forms (whole or ground) of flaxseed might have affected the results.

In the present study, the yolk index was positively affected by the consumption of whole or ground flaxseed

regardless of the consumption of the *ad libitum* grit. Similarly, Al-Hassani et al. [28] reported that a diet containing 6% flaxseed increased the total means of the height of egg yolk ($p < 0.01$); however, it did not affect the egg yolk percentage significantly. Kamanlı and Türkoğlu [29] declared that yolk index needs to be higher than 46.00. Egg yolk consists of light and dark yellow layers. The light yellow forms the chick in the hatchery, while the dark yellow feeds the chick [30]. The yolk index of all groups given flaxseed was higher than 46.47, and it was significantly higher than that of the control group (44.88).

Kirubakaran et al. [31] found that a diet containing flaxseed and sardines given to layers has no effect on their shape index, albumen index, yolk index, and Haugh unit of eggs. Similarly, research on broiler hens revealed that a diet containing high levels of n-3 fatty acids (3.50% fish oil) did not affect their Haugh unit of eggs [32]. Whole or milled flaxseeds did not affect the Haugh unit of the eggs [23]. These results are similar to that of the present study. However, Al-Hassani et al. [28] reported that a diet containing flaxseed increased the Haugh unit of eggs in quails.

In the present study, the analyses of flaxseed fatty acid and egg yolk pools of the trial groups showed that eicosapentaenoic acid (EPA) levels were numerically increased by the supplementation of flaxseeds. Several recent studies have shown that different amounts (5%, 7.5%, 15%, and 22%) of diet containing flaxseed given to layers increased omega-3 fatty acids in their egg yolk [33–35]. Generally, because of the high omega-3 content of flaxseed, diets containing flaxseed decrease blood cholesterol levels in quails, as Basmacioğlu et al. [36] found. Basmacioğlu et al. [5] declared that serum cholesterol level was significantly decreased by 8.64% diets containing flaxseed in laying hens; however, triglycerides level was not affected.

A diet containing 9% milled flaxseed decreased blood cholesterol levels in laying hens [23]. These results are contrary to the results of the present study. However, there are no significant differences obtained from quails in terms of their blood parameters like hematocrit, serum cholesterol, and serum triglycerides levels in the present trial. The difference may be caused by the use of different raw materials in the rations in the studies. The blood parameters of quails could possibly change due to the animals' aging. At the beginning of the laying period, quails (5-week-old) were used in the present trial.

Consumption of whole flaxseed was not altered by *ad libitum* consumption of limestone grit. The fact that the

feed efficiency did not change in the study suggests that the consumption of *ad libitum* grit does not affect the ability of quails to digest a diet containing 10% flaxseed.

On the other hand, a trial reported that hens that consumed 60% whole barley-based ration with insoluble grit (4 g/hen per week) had reduced egg production and feeding efficiency compared to hens that consumed ground barley [37]. However, more recently, De Witt et al. [38] studied the effects of particle size of limestone on hens and noted that production parameters were not affected by different sizes of (1.0 mm to 3.8 mm) calcitic particles. An investigation on broilers also showed that the ratio of diet containing 0.75% grits increased feed consumption and feed efficiency of local chickens under intensive conditions [10]. It is clear that the amount of consumed grit affects the performance of chickens. Kim and Guo [39], reported that a basal diet with a mixture of two different particles sized limestone (8% large and 2% small) increased feed consumption in laying hens compared to the diet containing single sized limestone (10%). This also shows that the size of consumed grit can affect layers. The differences between the results of the researches may stem from the animal species [37,38] subject to research, age of layers, and composition and dimensions of the gritstone [38,39].

5. Conclusions

For an important egg quality parameter, yolk index, it is clear that a diet containing flaxseed improved the inner quality of egg whether it is whole or grounded. On the other hand, birds can better digest a diet containing whole seeds with a grit diet. In addition to this, some of the previous studies noted that the size of grit stones was effective on quails' performance. However, in the present study, the investigated parameters of the groups that consumed and those that did not consume *ad libitum* grit with whole flaxseeds were not affected. This may be caused by the selected dietary seed species and the nutritional value of flaxseeds.

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