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Effect of Dietary Alfalfa Meal on Performance, Egg Quality, Egg Yolk Cholesterol and Hatchability Parameters of Quail Breeders

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A B S T R A C T

The aim of this study was to investigate the effects of different levels of alfalfa meal on performance, egg quality, egg yolk cholesterol and hatchability parameters of quail breeders. In this trial a total of 90 Japanese quail breeders (30 males, 60 females), 10 weeks old was distributed 5 experimental groups randomly. Each experimental group consisted of 6 replicates of 3 quails (1 males, 2 females) in each. During the 12 weeks experiment period, birds were fed with 5 dietary groups based on corn and soybean meal and containing 0 (control), 10, 20, 40 and 80 g/kg alfalfa meal. Feed and water were offered *ad-libitum* throughout the experiment. The different dietary levels of alfalfa meal had no significant effect on body weight change, egg production, egg weight, egg mass, feed conversion ratio, egg shell breaking strength, egg yolk triglyceride, fertility, hatchability of fertile eggs or hatchability of eggs set. The feed intake was decreased with 10 or 20 g/kg alfalfa meal levels in the diet. The eggshell weight was best in quails fed 20 g/kg alfalfa meal, and the egg yolk cholesterol was the lowest in quails fed 40 g/kg alfalfa meal. According to the results of this study the addition of 40 g/kg alfalfa meal in laying quail diets decreased the egg yolk cholesterol levels without adverse effect on performance.

Introduction

Alfalfa is high in fiber content, and is most often added to poultry diets as a source of xanthophylls for pigmentation, or as a source of so-called unidentified growth factors (Leeson and Summers, 2005). Dehydrated alfalfa is usually used at a very low levels in poultry diets, due to its high crude fiber and low metabolic energy contents (Dansky, 1971); however, it is a rich source of vitamins, carotenoids (Sen et al., 1998) and saponins (Whitehead et al., 1981). Heywang (1950) reported that addition of alfalfa meal to the layer diets at the levels of 50, 100, 150 and 200 g/kg had no effect on feed intake (FI), but an addition of more than 5% decreased egg production (EP). Güçlü et al. (2004) indicated that addition of 90 g/kg alfalfa meal into the laying quail diets had no adverse effect on performance and increased some of egg quality parameters. However, Mourao et al. (2006) reported that inclusion of alfalfa in diets of laying hens reduced performance expressed in terms of body weight and egg mass (EM). Tkacova et al. (2011) also reported that addition of high doses (60 g/kg) of alfalfa had lower feed conversion in broiler.

Alfalfa also contains high levels (2 to 3% dry matter) of saponins, which have showed hypocholesterolemic, anticarcinogenic, anti-inflammatory, and antioxidant activity (Rao and Gurfinkel, 2000; Francis et al., 2002). Reshef et al. (1976) showed that parameters of lipid metabolism were not affected by alfalfa meal saponins, in addition to increased cholesterol biosynthesis in liver. Saponin is hypocholesterolemic due to formation of unabsorbable cholesterol-saponin complexes in the intestinal absorption site, thereby lower absorption of exogenous and endogenous cholesterol or increasing the fecal excretion of bile acids (Sim et al., 1984; Sidhu and Oakenfull, 1986; Jenkins and Atwal, 1994). Story and Kritchevsky (1976) founded that alfalfa meal is more effective than other sources of cellulose binding bile acids. Turk and Barnett (1972) and McNaughton (1978) reported that alfalfa meal in the diet decreased egg yolk cholesterol levels. Similarly, Güçlü et al. (2004) showed that the addition of 90 g/kg alfalfa meal to laying quail diets decreased serum lipids and egg yolk cholesterol levels without adverse effect on performance. In contrast, some investigators reported that alfalfa meal had no effect on egg yolk cholesterol (Nakaue et al., 1980; Sim et al., 1984; Mourao et al., 2006).

As well as the limited number of studies on the effects

of alfalfa meal in poultry, there is no study investigating the effects of alfalfa meal on reproductive performance of poultry. The influence of alfalfa meal on poultry breeder is also unclear. Therefore, this experiment was conducted to determine the effects of alfalfa meal on performance, egg quality, egg yolk cholesterol and hatchability parameters in quail breeders.

Materials and Methods

A total of 90 Japanese quail breeders (30 males, 60 females), 10 weeks old were distributed 5 experimental groups randomly. There were 6 replicates in each experiment group and in each replicates there were 3 (1 males, 2 females) birds. During the 12 weeks experiment period, birds were fed with 5 dietary groups based on corn and soybean meal and containing 0 (control), 10, 20, 40 and 80 g/kg alfalfa meal. Experimental diets were balanced to meet or exceed the nutrient requirements for Japanese quail breeder (NRC, 1994) and formulated to be isocaloric and isonitrogenous with only the alfalfa meal levels in the diets differing (Table 1). Birds were housed in a breeder house equipped with 30 metal battery cages (30x45x25 cm). Feed and water were offered *ad-libitum* throughout the experiment (10-22 weeks). Light was provided for 16 h/day from 06:00 to 22:00 h throughout the experimental period. Quails were placed environmentally controlled room (23-25°C).

Body weight was obtained by weighting quails at the beginning and at the end of the experimental period. Egg production was recorded daily. Feed intake and egg weight (EW) were recorded biweekly. Egg mass was calculated from collecting data of EP and EW at biweekly via the following formula:

EM=(EP x EW)/ Periods (days)

Feed conversion ratio (FCR, g feed/ g egg) was calculated via the following formula:

FCR=FI(g of feed/quail/period)/EM(g of egg/quail/period)

The eggshell quality characteristics (shell breaking strength and shell weight) were determined with all collected eggs produced at the last two days of the each of the two weeks periods during the whole experiment. Eggshell breaking strength was measured using a cantilever system by applying increased pressure to the broad pole of the shell using an instrument (Egg Force Reader, Orka Food Technology, Israel). Eggs were then broken, and eggshell, albumen, and yolk were separated and weighed. Eggshells were weighed using a 0.001g precision scale. Eggshell weight was calculated via the following formula:

Eggshell weight(% of EW)=[Eggshell weight (g)/EW(g)x100]

Twelve eggs from each group (2 eggs from each replicate) were collected at the end of the experiment to determine egg yolk total cholesterol and triglyceride levels. The egg yolk total cholesterol and triglyceride levels were determined by using the methods of Berrio and Hebert (1990), Hammad et al. (1996), with slight modifications by Eren and Uyanik (2007) and Boehringer Mannheim GmbH Biochemica (1989). The eggs were hard-boild for 15 min, then the yolks were separated and 0.1 g samples of yolks were weighed accurately. Yolk lipids were extracted with isopropanol (4 ml/0.1 of yolk), then vortex-mixed and centrifuged at 3000 rpm for 5 min. The yolk lipids determined in the samples filtered by spectrophotometer using a commercial kit (HUMAN Cholesterol liquicolor for cholesterol, HUMAN Triglucerides liquicolor^{mono} for triglyceride).

Table 1 Composition of experiment diets

Ingredients	Alfalfa Meal, g/kg				
g/kg	Control	10	20	40	80
Corn	550.0	542.0	534.0	515.0	485.0
Soybean meal	269.0	274.0	279.0	283.0	303.0
Sunflower meal	70.0	60.0	50.0	40.0	00.0
Alfalfa meal	0.0	10.0	20.0	40.0	80.0
Vegetable oil	35.6	39.0	42.2	48.4	60.0
Limestone	55.2	54.8	54.4	53.2	51.4
Dicalcium Phosphate	13.0	13.0	13.1	13.1	13.2
Salt	3.5	3.5	3.5	3.5	3.5
Premiks[1]	2.5	2.5	2.5	2.5	2.5
DL-Methionine	1.2	1.2	1.3	1.3	1.4
Total	1000.0	1000.0	1000.0	1000.0	1000.0
Calculated nutrients					
Metabolisable Energy, kcal/ kg ME	2900	2902	2903	2901	2899
Crude Protein, g/kg	200.1	200.0	200.0	200.3	200.2
Calcium, g/kg	25.00	25.02	25.06	25.00	24.97
Available P, g/kg	3.51	3.50	3.51	3.50	3.48
Crude Cellulose, g/kg	38.38	39.58	40.78	44.00	48.83
Lysine, g/kg	10.53	10.52	10.51	10.44	10.39
Methionine, g/kg	4.55	4.51	4.57	4.49	4.42
Methionine + Cystine, g/kg	8.08	7.97	7.97	7.82	7.52

[1]Premix provided/kg of diet; Mn: 60 mg; Fe: 30 mg; Zn: 50 mg; Cu: 5 mg; 1, 1.1 mg; Se: 0.1 mg, Vitamin A, 8.800 IU; Vitamin D_3, 2.200 IU; Vitamin E, 11 mg; Nicotine acid, 44 mg; Cal-D-Pan, 8.8 mg; Riboflavin 4.4 mg; Thiamin 2.5 mg; Vitamin B_{12}, 6.6 mg; Folic acid, 1 mg; D-Biotin, 0.11 mg; Choline: 220 mg

Fertility and hatchability were determined by collecting eggs between the 11th and the 12th weeks of the experiment. Eggs were incubated at a temperature of 37.7°C with 65% relative humidity for 14 d. They were then transferred at random to hatcher trays and were maintained at 37.2°C and 75% relative humidity until hatching. The numbers of hatched chicks were counted after 18 d of incubation then fertility, hatchability of fertile eggs and hatchability of set eggs in the treatment groups were also determined (Aygun et al., 2012).

Data were subjected to ANOVA by using One-Way in Minitab (2000). Duncan's multiple range tests were applied to separate means (Duncan, 1955). Statements of statistical significance were based on a probability of $P<0.05$.

Results and Discussion

The body weight change (BWC), EP, EW, EM, FI and FCR are presented in the Table 2. In the present study, there were no significant differences in BWC, EP, EW, EM or FCR due to dietary alfalfa meal levels ($P>0.05$). There were significant reduction in the feed intake in the 10 or 20 g/kg alfalfa meal including group ($P<0.05$); however, no significant differences were observed at higher dietary alfalfa meal levels (40 and 80 g/kg) when compared with the control group.

The egg breaking strength, eggshell weight, egg yolk cholesterol and triglyceride, fertility, hatchability of fertile eggs and hatchability of eggs set are presented in the Table 3. The levels of dietary alfalfa meal had no significant effect on egg breaking strength, egg yolk triglyceride content and characteristic of hatchability ($P>0.05$). Dietary levels of alfalfa meal had a significantly effect on eggshell weight and egg yolk cholesterol content. The lowest eggshell weight ($P<0.05$) and egg yolk cholesterol content ($P<0.01$) were obtained from 40 g/kg alfalfa meal including diet.

According to the results of the present study, the dietary alfalfa meal had no significant effect on BWC, EP, EW, EM and FCR; but had a significant effect on FI ($P<0.05$). In earlier studies had shown that effects of alfalfa meal on performance could be different. Güçlü et al. (2004) reported that quail diets containing 30, 60 and 90 g/kg alfalfa meal had no effects on performance parameters. Similar results have also been reported by Khajali et al. (2007). Sim et al. (1984) observed that the birds fed with saponin at 0.2 g/kg or higher level in the diet were significantly decreased egg production and feed intake in laying hens. Mourao et al. (2006) showed that inclusion of alfalfa (151 g/kg) in diets of laying hens decreased body weight at week 44, 48, and 52 and FI in the first 4 weeks of the experiment. In addition, they reported that the presence of alfalfa meal in the diet reduced EP, EW, and consequently the obtained EM.

Some of the previous studies showed that different levels of dietary alfalfa meal were not affected eggshell quality. Mourao et al. (2006) observed that alfalfa meal was not affected the eggshell quality such as the rate cracked eggs, shell thickness, or egg specific gravity. Also, Khajali et al. (2007) reported that alfalfa meal in the laying hen diets had no significant effects on eggshell thickness and eggshell breaking strength. These results can be said to be similar to the results of the present study. In the present study, the eggshell weight was significantly lower fed with the 40 g/kg alfalfa meal in diets than 20 or 80 g/kg alfalfa meal in diet; however, no significant differences were observed with alfalfa meal groups when compared with the control group. Otherwise, Güçlü et al. (2004) showed that 60 and 90 g/kg alfalfa meal in the quail hens diet increased specific gravity of whole egg and eggshell thickness.

Table 2 Effects of different levels of alfalfa meal on performance of quail breeders

	Alfalfa meal, g/kg				
	Control	10	20	40	80
BWC, g	7.06±6.23	-7.22±2.36	-2.47±4.62	-12.56±8.92	-0.50±3.72
EP, %	90.51±0.95	89.82±0.86	89.57±0.36	88.05±0.93	90.70±0.34
EW, g	12.27±0.29	12.41±0.19	12.41±0.26	12.58±0.21	12.34±0.20
EM, (g/quail/d)	11.11±0.29	11.15±0.17	11.11±0.24	11.08±0.24	11.19±0.19
FI, (g/quail/d)	27.19 ± 0.36^{a}	25.64 ± 0.41^{bc}	25.24 ± 0.42^{c}	26.11 ± 0.44^{abc}	26.81 ± 0.46^{ab}
FCR, (g feed/g egg)	2.45±0.049	2.30±0.049	2.28±0.044	2.36±0.077	2.40±0.062

*Different superscripts [a, b, c] indicate significant differences ($P<0.05$) according to the dietary alfalfa meal levels.

Table 3 Effects of different levels of alfalfa meal on egg quality and hatchability of quail breeders

	Alfalfa meal, g/kg				
	Control	10	20	40	80
Eggshell Quality					
Egg breaking strength, kg	1.61±0.06	1.66±0.04	1.71±0.03	1.62±0.04	1.59±0.06
Eggshell weight, %	8.42 ± 0.13^{ab}	8.35 ± 0.07^{ab}	8.55 ± 0.08^{a}	8.14 ± 0.09^{b}	8.52 ± 0.09^{a}
Egg yolk					
Cholesterol, mg/dl	147.1 ± 1.22^{A}	145.8 ± 2.00^{A}	144.7 ± 1.26^{AB}	139.9 ± 0.77^{B}	142.6 ± 1.12^{AB}
Triglyceride, mg/dl	109.1±1.77	108.0±0.76	108.0±1.13	109.3±2.29	110.9±2.11
Hatchability					
Fertility, %	97.11±1.94	93.89±3.08	93.33±5.44	92.45±2.85	89.58±6.10
Hatchability of fertile eggs, %	78.29±5.39	77.74±6.08	84.98±4.03	82.50±4.00	81.35±6.03
Hatchability of eggs set , %	75.86±4.93	73.09±6.48	78.98±5.50	76.60±5.39	71.68±4.85

*Different superscripts [A, B] indicate significant differences ($P<0.01$) according to the dietary alfalfa meal levels.
*Different superscripts [a, b] indicate significant differences ($P<0.05$) according to the dietary alfalfa meal levels.

The present study, the egg yolk cholesterol was significantly lower in the 40 g/kg alfalfa meal including diet when compared with control or 10 g/kg alfalfa meal including groups. McNaughton (1978) observed that the alfalfa meal in the laying hens decreased the egg yolk cholesterol. Similarly, Güçlü et al. (2004) demonstrated that a significant decrease in the egg yolk cholesterol by feeding alfalfa meal in quails. Similar results have also been reported by Turk and Barnett (1972) and Khajali et al. (2007). On the other hand, these results do not agree with some of investigators who observed that alfalfa meal had no effect on egg yolk cholesterol (Mourao et al., 2006) or saponin (Sim et al., 1984) in laying hens.

Conclusion

Inclusion of 10 or 20 g/kg alfalfa meal levels in the diets of quail breeders reduced FI, but it is no effect on others performance parameters. Forty g/kg of alfalfa meal in quail breeder diets decreased egg yolk cholesterol. Addition of alfalfa meal to quail breeder diets slightly decreased fertility; however, it is slightly increased hatchability of fertile eggs.

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