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Walnut Meal as an Excellent Source of Energy and Protein for Growing Japanese Quails

Research Article

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ABSTRACT

The present study was designed to study the chemical composition, apparent and true metabolizable energy values of the walnut meal and to evaluate the effects of different levels of walnut meal (0, 10, 20 and 30%) on Japanese quail's growth performance, blood metabolites, relative weight of different organs, malondialdehyde (MDA) concentration in breast meat and egg yolks' cholesterol. This study was conducted as a completely randomize design with 288 unsexed Japanese quails randomly dividing into 4 treatments with 4 replicates of 18 birds each. As a result of this study, no significant differences were found for feed intake and feed conversion ratio ($P>0.05$), except the birds fed 30% walnut meal showed lower weight gain compared to the control at 7-21 days of age ($P<0.05$). There were no significant differences in serum glucose, uric acid, serum aspartate aminotranspherase (AST) and alanine aminotranspherase (ALT) activities between different dietary treatments. The serum low density lipoprotein (LDL), cholesterol and triglyceride tended to decrease linearly ($P<0.01$) as the walnut meal levels were increased. The serum high density lipoprotein (HDL) level in quails fed 10% walnut meal were significantly higher than control group ($P<0.05$). Consumption of different levels of walnut meal significantly decreased malondialdehyde (MDA) concentration in breast meat of chicks aged 42 d ($P<0.01$). Different dietary treatments had no effect on the relative weight of different organs and carcass traits. In general, walnut meal is a good source of energy (apparent metabolizable energy corrected for nitrogen (AMEn) 3689 kcal/kg), oil (23%) and crude protein (40%) and could be used up to 20% for young chicks and 30% for older chicks, without any adverse effect on growth performance.

KEY WORDS growth performance, Japanese quail, meat quality, walnut meal.

INTRODUCTION

According to the Food and Agriculture Organization (FAO, 2012), the top 3 walnut (*Juglans regia*) producers in 2012 respectively were China, the United States and Iran. Walnut with 64000 hectares cultivated area is one of the important horticulture products in Iran. The walnut seed (kernel) represents from 40 to 60% of the nut weight, depending mainly on the variety. The seed has high levels of oil (52-70%) in which polyunsaturated fatty acids predominate (Prasad, 2003; Martinez *et al.* 2006). In addition to oil, walnuts provide appreciable amounts of proteins (up to 24% of the walnut seed weight), carbohydrates (12-16%), fibre (1.5-2%) and minerals (1.7-2%) (Savage, 2001; Prasad, 2003). According to USDA (2013), dry matter, ether extract, crude protein, ash and crude fiber content of the nut are 95.3, 49.42, 21.22, 2.99 and 12.2%, respectively. A lot of studies have been undertaken regarding the health effects of walnut in human nutrition. Walnut and almond can reduce serum low density lipoprotein (LDL), increase

serum high density lipoprotein (HDL) and thus, can reduce coronary heart disease risk factors (Jenkins *et al.* 2002; Hyson *et al.* 2002; Kalgaonkar *et al.* 2011). In the past decades, different phenolic compounds with antioxidant activities were characterized and identified in walnut seed extract and its skin, shell and hull as walnut by-products (Fukuda *et al.* 2003; Wijerant *et al.* 2006; Labuckas *et al.* 2008; Oliveira *et al.* 2008).

Walnut meal, a by-product of walnut processing, is obtained after oil has been extracted from the walnut kernels, mostly by cold pressing methods which leaves considerable amount of oil in walnut meal. There is no available report regarding the use of walnut meal in poultry diets. This study was planned to evaluate the effects of using different levels of walnut meal on Japanese quail's growth performance, blood metabolites, relative weight of different organs and egg yolks' cholesterol.

MATERIALS AND METHODS

Metabolizable energy assay

The experiment was conducted at the Shahid Bahonar University of Kerman, Kerman, Iran. In the first trial, apparent metabolizable energy (apparent metabolizable energy (AME) and apparent metabolizable energy corrected for nitrogen (AMEn)) content of walnut meal was determined using total collection method (Macleod, 2002). Walnut meal was substituted with a corn-soybean meal basal diet at 40% level and then AME and AMEn of this experimental diet and basal diet were determined. Twelve adult leghorn cockerels (165 d old) with mean body weight of 1645 ± 30 g were housed in individual cages, with 6 pens per treatment. Both feed (as mash) and water were provided *ad libitum*. After 5 days adaptation to experimental diets, excreta were collected and corresponding feed intake recorded for subsequent three days. Feed was removed overnight at the start and termination of the excreta collection period. The collected excreta were dried at 65 ˚C for 48 hours in a forced-air oven. Then samples were placed in the laboratory environment for 24 hours to equilibrate with ambient humidity and then were ground before dry matter, gross energy, and nitrogen determinations. All samples were analyzed in duplicates. The ME value of walnut was determined according to the formula:

$$ED = (P \times EF) + (1-P)EB$$

Where:
ED: ME of the experimental diet.
P: level of date palm in the experimental diet.
EF: ME of date palm.
(1-P): level of basal diet in experimental diet.
EB: ME of basal diet (Marquardt, 1962).

In the second trial, twelve adult leghorn cockerels with uniform body weights were used to determine the true metabolizable energy using Sibbald method (Sibbald, 1986).

Birds fasted for 48 hours, then 6 birds received 25 g walnut meal using force feeding procedure and their excreta were collected for 48 hours. Also six cockerels were used fasted to determine the endogenous urinary energy and fecal metabolic energy losses. Finally, true metabolizable energy (TME) and true metabolizable energy corrected for nitrogen (TMEn) were calculated. Chemical compositions of walnut meal and excreta samples were measured according to the prevalent methods (AOAC, 2005).

Quail assay

A total of 288 day-old unsexed Japanese quail chicks were randomly allocated to 4 experimental groups with 4 replicates and 18 chicks in each. The diets were fed to quail chicks for 6 weeks. Four experimental diets were formulated to meet the NRC requirements (NRC, 1994) of quails as shown in Table 1.

All experimental diets were formulated and adjusted to be isonitrogenous and isocaloric. Diets contained four levels of walnut meal (0, 10, 20 and 30%). Feed and water were provided on an *ad libitum* basis. Lighting program was adjusted to meet 24 hours of light daily. Body weight gain and feed intake were determined on a weekly basis.

To measure the weight of different organs, two male chicks from each replication were selected randomly and sacrificed at 21 and 42 days of age. At 42 days of age, blood samples from 2 chicks per replicate were also taken from the neck vein and centrifuged at 3000 rpm for 15 minutes. All serum tests including LDL, HDL, serum aspartate aminotranspherase (AST) and alanine aminotranspherase (ALT) activities, uric acid, triglyceride, cholesterol and glucose were analyzed using commercial Pars-Azmoon kits and an auto analyzer.

Malondialdehyde (MDA) concentration in breast meat (fresh and/or frozen at -20 ˚C for 30 days) was also determined (Tarladgis *et al.* 1960).

Breast meat pH was also determined by blending 10 g sample in 100 mL distilled water for one minute and pH was measured using a pH meter (model AZ 86502) (Ensoy *et al.* 2004). At the beginning of laying, three eggs per replicates were selected and total cholesterol content in the yolks were determined according to the method of Pasin *et al.* (1998).

Statistical analyses

This experiment was performed as a completely randomized design. The data were analyzed using the general linear (GLM) procedure of SAS (SAS, 2003) and Duncan's multiple range test was used to detect differences among treatment means ($P<0.05$).

Table 1 Diet formulation and calculated chemical composition of diets (as fed)

Ingredients (%)	Level of walnut meal (%)			
	0 (control)	10	20	30
Corn	43.30	41.63	39.97	38.20
Barley grain	0.20	1.47	2.73	4
Soybean meal	47.79	38.13	28.5	18.92
Walnut meal	0	10	20	30
Soybean oil	5.44	4.29	3.15	2
Calcium carbonate	1.35	1.40	1.40	1.50
Dicalcium phosphate	0.8	0.60	0.40	0.20
Common salt	0.37	0.37	0.37	0.37
DL-methionine	0.15	0.19	0.22	0.26
L-lysine hydro chloride	0	0.25	0.50	0.75
Vitamin premix[1]	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25
Grit	0.1	1.17	2.21	3.3
Chemical composition				
ME (kcal/kg)	3000	3001	3002	3000
Crude protein (%)	24.82	24.82	24.82	24.84
Lysine (%)	1.39	1.388	1.377	1.368
Methionine (%)	0.522	0.531	0.541	0.551
Methionine + cysteine (%)	0.915	0.871	0.828	0.784
Calcium (%)	0.839	0.837	0.836	0.834
Available phosphorus (%)	0.315	0.319	0.319	0.318
Sodium (%)	0.158	0.157	0.157	0.156
Crude fiber (%)	4.3	4.36	4.42	4.48

[1] Provided the following per kg of diet: Retinol acetate: 3.1 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Niacin: 30 mg; Pantothenic acid: 10 mg; Pyridoxine: 3 mg; Folic acid: 1 mg; Cyanocobalamine: 15 µg; Biotin: 0.1 mg; Cholecalciferol: 0.05 mg; Alpha-tocopherol acetate: 18 mg; Menadion: 2 mg and Choline chloride: 0.4 g.
[2] Fe: 50 mg; Mn: 100 mg; Zn: 85 mg; Cu: 10 mg; Se: 0.2 mg and I: 1 mg.

RESULTS AND DISCUSSION

Dry matter, ether extract, crude fibre, crude protein, ash, calcium, total phosphorus and sodium content of walnut meal were 95, 23, 6.3, 40, 5.5, 5.2, 0.67 and 0.2 %, respectively. AME, AMEn, TME and TMEn of walnut meal were 3478.24 ± 77, 3689.42 ± 71, 3997.46 ± 53 and 3673.83 ± 55 (kcal/kg, as fed basis), respectively.

According to USDA (2013), dry matter, ether extract, crude protein, ash and crude fiber content of walnut meal are 95.3, 49.42, 21.22, 2.99 and 12.2%, respectively. So, this nut has high levels of oil in which polyunsaturated fatty acids predominate (Prasad, 2003; Martinez *et al.* 2006; Ozcan *et al.* 2010). The chemical composition of walnuts in different reports is not the same. It seems walnuts provide appreciable amounts of proteins (up to 24% of the walnut meal weight), carbohydrates (12-16%) and minerals (1.7-2%) (Savage, 2001; Prasad, 2003).

There is no available report regarding the chemical composition and ME content of walnut meal. Considering walnut meal composition, it should be an appropriate source of energy and protein.

Performance parameters and carcass traits

The effects of different levels of walnut meal on feed intake, weight gain and feed conversion ratio of Japanese quails are shown in Table 2. During the whole experimental period extended from 1-42 days of age the obtained results showed that growth performance of the birds fed diets with different levels of walnut meal were not statistically different compared to control, although the birds fed 30% walnut meal showed lower weight gain as compared with the control and 10% walnut meal at 7-21 days of age ($P<0.05$). Thus it seems that walnut meal should be limited to 20% for young chicks.

There is no report regarding the effect of walnut meal on poultry growth performance. However, recent results have shown that the encapsulation of intracellular lipids by the cell walls of almond, restricts their digestion in the stomach and small intestine and therefore available for fermentation in the colon by the gut microbiota (Mandalari *et al.* 2008a; Mandalari *et al.* 2008b). These results should be explained the poorer performance of the younger chicks fed 30% walnut diet.

The effects of different dietary treatments on the relative weight of different internal organs and carcass traits at 21 d and 42 d of age are shown in Tables 3 and 4 and found no significant effects among the groups.

Blood analysis

The effect of different dietary treatments on some blood serum metabolites at 42 days of age are presented in Table 5. There were no significant differences in glucose, uric acid, AST and ALT levels between different dietary treatments. The serum LDL, cholesterol and triglyceride tended to decrease linearly ($P<0.01$) as the walnut meal levels were increased. The serum HDL level in quails fed 10% walnut meal were significantly higher than control group ($P<0.05$). The results presented here are in agreement with the previous reports showing that the application of walnut and almond in human diets can reduce blood total cholesterol and LDL and also can increase blood HDL (Hyson *et al.* 2002; Jenkins *et al.* 2002; Kalgaonkar *et al.* 2011). These effects of walnuts are mediated by components in the oil fraction of this nut (Hyson *et al.* 2002) or probably in part because of the nonfat (protein and fiber) and monounsaturated fatty acid components of the nut (Jenkins *et al.* 2002).

Meat quality

The effects of walnut meal on fresh and frozen breast meat quality and egg yolk cholesterol are given in Table 6. There were no significant differences in pH and CP content of fresh breast meat and egg yolk cholesterol concentration between different dietary treatments.

Table 2 The effects of different levels of walnut meal on feed intake (FI, g/bird/day), body weight gain (WG, g/bird/day) and feed conversion ratio (FCR) of Japanese quails

Walnut meal level (%)	7-21 (day)			21-42 (day)			7-42 (day)		
	FI	WG	FCR	FI	WG	FCR	FI	WG	FCR
0 (control)	14.72	6.79[a]	2.16	26.48	5.03	5.25	21.78	5.73	3.79
10	14.63	6.74[a]	2.17	25.75	5.40	4.78	21.30	5.94	3.59
20	14.64	6.61[ab]	2.21	25.98	5.18	5.04	21.45	5.75	3.73
30	14.40	6.28[b]	2.29	25.57	5.14	4.99	21.10	5.60	3.76
SEM	0.34	0.12	0.04	0.60	0.16	0.21	0.42	0.10	0.10
P-values	0.917	0.045	0.176	0.73	0.49	0.51	0.72	0.18	0.55

The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effects of different dietary treatments on the relative weight of different organs and carcass traits in Japanese quails aged 21d (% of live body weight)

Walnut meal level (%)	Heart	Liver	Spleen	Large intestine	Small intestine	Ceca	Bursa of fabricius	Pancreas	Thighs	Breast
0 (control)	0.73	2.42	0.06	0.98	2.22	0.71	0.11	0.28	13.15	21.59
10	0.73	2.48	0.07	0.94	2.03	0.68	0.11	0.20	12.94	23.07
20	0.73	2.36	0.05	0.81	1.53	0.64	0.10	0.28	14.03	22.97
30	0.61	2.51	0.06	0.75	1.56	0.56	0.08	0.29	13.73	22.79
SEM	0.049	0.090	0.007	0.081	0.275	0.113	0.011	0.035	0.395	0.560
P-values	0.27	0.68	0.59	0.16	0.22	0.81	0.25	0.29	0.20	0.23

SEM: standard error of the means.

Table 4 The Effects of dietary treatments on the relative weight of different organs and carcass traits in Japanese quails aged 42 d (% of live weight)

Walnut meal level (%)	Heart	Liver	Spleen	Large intestine	Small intestine	Ceca	Bursa of fabricius	Pancreas	Thighs	Breast
0 (control)	0.82	1.39	0.038	0.58	1.25	0.45	0.085	0.18	13.89	25.31
10	0.78	1.43	0.033	0.62	1.43	0.54	0.052	0.18	13.95	23.63
20	0.74	1.61	0.068	0.64	1.47	0.50	0.052	0.18	12.85	24.05
30	0.80	1.30	0.055	0.53	1.33	0.57	0.09	0.29	14.34	25.35
SEM	0.049	0.104	0.005	0.043	0.101	0.058	0.013	0.025	0.623	0.495
P-values	0.4	0.85	0.21	0.88	0.67	0.55	0.38	0.59	0.83	0.42

SEM: standard error of the means.

Table 5 Effects of dietary treatments on blood serum parameters in Japanese quails aged 42 d

Walnut meal level (%)	LDL (mg/dL)	HDL (mg/dL)	AST (IU/L)	ALT (IU/L)	Uric acid (mg/dL)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	Glucose (mg/dL)
0 (control)	63.6[a]	60.1[b]	7.6	199	10.2	61.2[a]	136[a]	275
10	48.5[ab]	79.0[a]	8.7	230	12.0	65.0[a]	140[a]	249
20	37.1[b]	63.6[b]	9.5	198	11.4	55.7[a]	112[b]	253
30	32.2[c]	69.8[ab]	7.5	206	10.1	41.0[b]	110[b]	275
SEM	5.57	4.06	0.83	11.11	0.90	4.39	5.76	8.69
P-values	0.008	0.03	0.32	0.19	0.39	0.01	0.004	0.1

LDL: low density lipoprotein; HDL: high density lipoprotein; AST: aspartate aminotranspherase and ALT: alanine aminotranspherase.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 6 Effects of dietary treatments on breast meat quality and egg yolk cholesterol content in Japanese quails

Walnut meal level (%)	CP (%)	pH	MDA-42[1]	MDA-42[2]	MDA-21[3]	Cholesterol (mg/g yolk)
0 (control)	20.5	6.36	0.23	2.64[a]	0.88	13.54
10	21.21	6.36	0.21	0.95[bc]	0.69	14.08
20	21.48	6.41	0.21	0.78[c]	0.80	14.06
30	21.13	6.36	0.23	1.16[b]	0.84	13.47
SEM	0.429	0.018	0.024	0.097	0.046	0.34
P-values	0.45	0.29	0.79	0.0001	0.058	0.53

[1] Malondialdehyde concentration in fresh breast of chicks aged 42 d (mg MDA/kg).
[2] Malondialdehyde concentration in breast meat after 30 d freezing in chicks aged 42 d (mg MDA/kg).
[3] Malondialdehyde concentration in breast meat after 30 d freezing in chicks aged 21 d (mg MDA/kg).
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The MDA concentration in fresh breast meat of quails aged 42 d and in frozen meat of birds aged 21 d were not significantly affected by the dietary treatments. However, MDA concentration in breast meat after 30 d freezing in chicks aged 42 d was significantly affected by the dietary treatments. Consumption of different levels of walnut meal significantly decreased MDA concentration ($P<0.01$).

Some studies showed the scavenging activity of superoxide and hydroxyl radicals by different walnut extracts. Wijerant *et al.* (2006) showed brown skin extract at 50 ppm effectively inhibited copper-induced oxidation of human LDL cholesterol compared to whole seed and green shell cover extracts. Torabian *et al.* (2009) reported that the consumption of walnut seed increased serum polyphenol concentrations, increased total antioxidant capacity and reduced serum lipid peroxidation. The antioxidant activity of walnut has been attributed to its phenolic compounds (Fukuda *et al.* 2003; Wijerant *et al.* 2006; Labuckas *et al.* 2008; Oliveira *et al.* 2008).

CONCLUSION

The finding presented here provided new information for using walnut meal in quail diets. In general, walnut meal is a good source of energy (AMEn 3689 kcal/kg), oil (23%) and crud protein (40%) and could be used up to 20% for young chicks and 30% in finisher diets, without any adverse effect on growth performance.

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