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Original Research Article

Effects of Replacing Corn Grain by Reconstituted or Un-Reconstituted Sorghum Grain on Blood Biochemical Parameters in Japanese Quails

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ABSTRACT

Current study was carried out to evaluate effects of replacing un-reconstituted or reconstituted sorghum grain with corn grain on blood constituents in Japanese quail (*Coturnix coturnix japonica*). Seven hundred day-old unsexed quail chicks were fed basal diet and raised in cage system for 20 day. At day 21 quails were sexed and 300 male chicks reared for 21 day experimental period (21-42). Experimental birds were divided into 5 treatment groups (4 replicates contain 15 birds per pen). Experimental treatments were: (A) diet contains corn grain and without sorghum (control), (B) replacing 50% reconstituted sorghum, (C) replacing 50% un-reconstituted sorghum, (D) replacing 100% reconstituted sorghum, and (E) replacing 100% un-reconstituted sorghum grain in place of corn grain, respectively. A 6-h prior the end of the study birds were deprived from food. Blood samples were taken and serum cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride, total protein, urea, uric acid and glucose levels evaluated by colorimetric method using auto analyzer. According to the results, there was a significant difference on serum triglyceride, urea, total protein and glucose between experimental groups compared to control group (P< 0.05). These results suggest that replacing reconstituted sorghum had better effects than un-reconstituted sorghum on blood constituents in Japanese quails.

Keywords: Blood parameters, Corn grain, Japanese quails, Reconstituted, Sorghum

Mısır Yerine Sulandırılmış ve Sulandırılmamış Sorgum Kullanımının Japon Bıldırcınlarının Kan Parametreleri Üzerine Etkileri

ÖZ

Bu çalışma, sulandırılmış ve sulandırılmamış sorgum ve mısır tanelerinin, Japon Bıldırcını'nın (*Coturnix coturnix japonica*) kan bileşenleri üzerine etkisini incelemek amacıyla gerçekleştirilmiştir. 7 günlük çiftleşmemiş bıldırcınlar temel bir diyet ile beslenmiş ve 20 gün boyunca kafes içerisinde yetiştirilmiştir. 21. Günde bıldırcınlar çiftleştirilmiş ve 300 erkek yavru 21 gün boyunca beslenmiştir (21-42). Deney kuşları 5 adet muamele grubuna ayrılmıştır (Kafes başına 15 kuş içeren 4 tekerrür). Muameleler: (A) Sadece mısır içeren diyet (kontrol), (B) mısır yerine % 50 sulandırılmış sorgum, (C) % 50 sulandırılmamış sorgum, (D) % 100 sulandırılmış sorgum ve (E) % 100 sulandırılmamış sorgum şeklinde planlanmıştır. Çalışmanın bitmesine 6 saat kala kuşların beslenmesi kesilmiştir. Kuşlardan kan örnekleri alınıp serum kolesterol, yüksek yoğunluklu lipoprotein, düşük yoğunluklu lipoprotein, trigliserid, toplam protein, üre, ürik asit ve glukoz seviyeleri kolorimetrik yönteme göre analiz edilmiştir. Sonuçlara göre serum trigliserid, üre, toplam protein ve glukozda kontrol gruplarına göre istatistiksel olarak fark bulunmuştur. Sulandırılmamış sorgum yerine sulandırılmış sorgum kullanımı Japon Bıldırcını kan bileşenlerinde olumlu yönde etki etmiştir.

Anahtar Kelimeler: Kan parametreleri, Mısır, Japon bıldırcını, Sulandırılmış, Sorgum

INTRODUCTION

Sorghum grain (*Sorghum bicolor* L.) has expanded to become one the most important grain produced in variety of countries for poultry production. The feed value of sorghum is commonly less than corn and regularly less than wheat for starter broilers. Lower digestibility appears to occur because of the very tightly bound starch / protein matrix in the peripheral endosperm. This matrix does not readily break down by digestive enzymes in gastrointestinal tract (GIT) therefore they have to exposure longer time for proper digestion (Bryden *et al.* 2009). In this regards, Parthasarathy (2005) and Issa *et al.* (2007) reported that sorghum grains can play a significant role in poultry feeds. By contrast it is suggested sorghum could be a suitable feedstuff in the poultry industry (Dowling *et al*, 2002; Travis *et al*, 2006). Sorghum is sole among cereals in that produce large amounts of phenolic compounds (Cheeke 1998). Phenolic compounds (include tannins) contain one or more aromatic rings with one or more hydroxyl groups which enable formation of cross linkages with proteins and other

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macromolecules. Phenolic compounds are divided into several categories, including simple phenols, phenolic acids, hydrolysable tannins (HT), condensed tannins (CT), lignins and lignans (Hassanpour *et al.* 2011).

It is difficult to characterize phenolic compounds, including tannins in sorghum. Sorghum contains huge quantity of CT (Cheeke 1998). Tannins are distinguished from lignin by their relative solubility and ability to combine with protein to form leather-like precipitates which are resistant to attack by proteases or microbes (Van Soest 1994). Previously, Hahn *et al.* (1984) and Hagerman and Zheo (1997) have reviewed the methods available for sorghum phenol and tannin analysis and it is apparent that different methods measure different components of the phenolic complex. The essential property of tannins is their ability to combine with protein and other polymers such as cellulose, hemicelluloses and pectin to form stable complexes (Mangan 1988). Previously, capacity of high-tannin sorghums to depress growth performance in broilers has been demonstrated frequently and was reviewed by (Nyachoti *et al.* 1997). They have discussed that, responses to sorghum tannins are variable and are not closely correlated to bird performance, which suggests other factors may be involved in sub-optimal performance of birds offered sorghum-based diets. However, Marzoni *et al.* (2005) reported that dietary inclusion of quebracho (*Schinopsis lorentzii*) tannins had no significant effects on plasma total protein (TP) in pheasant females. Medugu *et al.* (2010) revealed that replacing maize with sorghum grain had no adverse effects on carcass and blood constituents of broiler chickens. Shawrang *et al.* (2011) stated that removal of undesirable components such tannins, is essential to improve the nutritional quality of sorghum grain. Kumar *et al.* (2007) indicated that reconstitution of sorghum is an effective process for improving its nutritive value. In this treatment, moisture content of grains rise to 25-30% and then storing in anaerobic conditions for three weeks. Manwar and Mandal (2009), also stated that reconstitution of sorghum may have beneficial effects on nutrient utilization in broiler chickens.

There is a little information on effects of reconstituted sorghum and tannin tolerance in Japanese quails (Ragab *et al.* 2002; Faquinello *et al.* 2004). Biological nature of Japanese quail lets us to thinking that it can tolerate more tannin than broiler chickens in their diet. Based on previous studies on effects of sorghum as tannin containing foodstuff on animals, our hypothesis was to clarify possible effects of replacing corn grain by RS or URS sorghum grain on blood cholesterol (Chl), high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), TP, urea, uric acid (UA) and glucose (Glu) levels in Japanese quails.

MATERIALS AND METHODS

Birds and diets

Seven hundred day-old mixed sex quail chicks (*Coturnix coturnix japonica*) were fed basal diet and raised in cage system for first 20 days after hatch. At day 21, quails were sexed based on their breast feather color and 300 male chicks with same weight selected and reared for 21 day experimental period (days 21-42). Experimental birds were divided into 5 treatment groups (4 replicates contain 15 birds per each pen). Treatment groups were received experimental diets: (A) diet contains corn grain and without sorghum grain (B) replacing 50% RS sorghum grain (C) replacing 50% URS sorghum (D) replacing 100% RS sorghum grain and (E) replacing 100% URS sorghum in place of corn grain, respectively. Diets were formulated using User Friendly Feed Formulation Done Again (UFFDA) (Pesti *et al.* 1992), according to nutritional suggestions. Composition of experimental diets is presented in Table 1.

Table 1. Feed composition and nutrient contents of experimental diets of Japanese quails at grower period (21- 42 d).

	Treatments				
	A	**B**	**C**	**D**	**E**
Ingredients	0% sorghum (C)	50% sorghum (RS)	50% sorghum (URS)	100% sorghum (RS)	100% sorghum (URS)
Corn grain	50	25	25	0	0
Sorghum grain	0	25	25	50	50
Wheat grain	6.80	8.04	8.04	8.01	8.01
Soybean meal	32.61	31.07	31.07	31.15	31.15
Gluten meal	7	7.62	7.62	7.70	7.70
Oyster shell	1.6	1.35	1.35	1.35	1.35
DCP	0.82	0.81	0.81	0.78	0.78
Lys. Sup.	0.25	0.18	0.18	0.19	0.19
Met. Sup.	0.1	0.11	0.11	0	0
Coccidiostat	0.07	0.07	0.07	0.07	0.07
Vit. Premixes	0.25	0.25	0.25	0.25	0.25
Min. Premixes	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Nutrients (calculated)					
ME (Kcal/Kg)	2900	2900	2900	2875.49	2875.49
CP %	23.74	23.50	23.50	23.50	23.50
CF %	3.68	3.64	3.64	3.67	3.67
Ca %	0.90	0.80	0.80	0.80	0.80
Avaiable P %	0.30	0.30	0.30	0.30	0.30
Met. %	0.50	0.50	0.50	0.40	0.40
Lys. %	1.30	1.20	1.20	1.20	1.20
Cys. %	0.39	0.39	0.39	0.39	0.39
Met. + Cys. %	0.89	0.89	0.89	0.79	0.79
Threonine %	0.87	0.86	0.86	0.86	0.86
Na %	0.12	0.12	0.12	0.11	0.11
K %	0.86	0.85	0.85	0.86	0.86
Cl %	0.19	0.21	0.21	0.22	0.22

Vit. = Vitamin premix, per kg of diet: vitamin A: 8500000 IU; vitamin D_3: 2500000 IU; vitamin E: 11000 IU; vitamin k_3: 2200 mg; Thiamine: 1477 mg; Riboflavin: 4000 mg; Pantothenic acid: 7840 mg; Pyridoxine: 7840 mg; B_{12}: 10 mg; Folic acid: 110 mg and Choline chloride: 400000 mg.

Min. = Mineral premix, per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 75000 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 74.4 mg; Zn (ZnO, 80.35% Zn): 64.675 mg; Cu ($CuSO_4$. $5H_2O$): 6000mg; I (KI, 58%I): 867 mg; Se (NaSeO3, 45.56% Se): 200 mg.

DCP= Di calcium phosphate, ME = Metabolizable energy, Lys = Lysine, Met = Methionine, Ca = Calcium, Cys = Cysteine, C: control

Nutrients calculated based on NRC (1994).

Sorghum preparation

Sorghum grains were purchased from farms of the Miyaneh region, East Azerbaijan, Iran. Half of grains reconstituted by adding water to whole grain to raise the moisture level to 30% (DM 70%), followed by anaerobic storage in sealed plastic buckets for 21 days at room temperature (25 ºC). Subsequently, the grains were sun-dried, until the moisture content reached 10%, and ground for use as Japanese quails diet (Kumar *et al.*, 2007).

Blood samples

A 6-h prior the end of the study birds were deprived from food (FD6). Blood samples were collected using disposable syringes. Blood samples were centrifuged at 750× g for 10 minute and plasma was separated and stored at -20 ºC until used. Serum Chl, HDL, LDL, TG, TP, urea, uric acid and Glu levels determined by colorimetric method using auto analyzer (Mindray- BS-200, Germany) at clinical pathology laboratory, at the Islamic Azad University, Shabestar Branch. Shabestar, Iran. All rearing and experimental processes performed

based on animal right and welfare protocol prepared by department of animal science, Islamic Azad University, Shabestar Branch in 1990.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) in a completely randomized design and treatment means were tested for statistical significance by Duncan's multiple range tests using SAS Statistical software (2007).

RESULTS AND DISCUSSION

Effects of replacing corn grain by RS or URS sorghum grain on blood parameters in Japanese quails in day 42 are presented in Table 2. According to the data there was a significant difference on serum HDL levels in Japanese quails. Replacing 50% RS significantly increased HDL levels compared to 100% URS ($P < 0.05$) but there was no significant difference compared to control group in day 42 ($P > 0.05$). There was no significant difference observed in serum LDL and Chl levels after replacing corn grain by RS or URS sorghum grain in Japanese quails ($P > 0.05$). A significant difference was observed in serum Glu levels after replacing 50% RS compared to 100% URS ($P < 0.05$) but was no significant difference detected compared to control group in day 42 ($P > 0.05$). There was a significant increase in serum TP levels after replacing corn grain by 100% RS compared to control group ($P < 0.05$). A significant reduction was observed in serum urea levels after replacing 100% URS and 100% RS compared to 50% RS ($P < 0.05$) but was no significant difference detected compared to control group ($P > 0.05$). No significant difference observed in uric acid levels after replacing corn grain by reconstituted or un-reconstituted sorghum grain in Japanese quails in day 42. It seems replacing RS sorghum had better effects in experimental birds. Also, it showed similar results to control group compared to URS groups.

Table 2. Effects of replacing corn grain by reconstituted or un-reconstituted sorghum grain on blood biochemical parameters in Japanese quails at 42 days of age.

Treatments	LDL (mg/dl)	HDL (mg/dl)	Chl (mg/dl)	TG (mg/dl)	Glu (mg/dl)	TP (g/l)	Urea (mg/dl)	Uric acid (mg/dl)
A (0% S)	63.66	42.00^{ab}	196.14	98.25^{a}	313.25^{a}	17^{b}	2.67^{ab}	5.43
B (50% RS)	78.00	49.37^{a}	216.38	97.75^{a}	306.38^{a}	19^{b}	3.27^{a}	4.44
C (50% URS)	65.25	44.37^{ab}	183.13	75.37^{b}	282.75^{ab}	16^{b}	2.60^{ab}	4.65
D (100% RS)	69.00	48.12^{a}	202.71	89.50^{a}	303.38^{ab}	26^{a}	2.41^{b}	4.72
E (100% URS)	55.37	36.50^{b}	162.63	73.75^{b}	266.00^{b}	14^{b}	2.10^{b}	3.97
P value	0.0995	0.0488	0.0778	0.0313	0.0086	0.0071	0.0484	0.3484
SEM	5.61	3.13	13.35	6.82	9.72	0.20	0.26	0.48

S: sorghum RS: reconstituted sorghum; URS: un-reconstituted sorghum. Chl: cholesterol, TG: triglycerides, Glu: glucose, TP: total protein. SEM: standard error of means.

There is significant differences between groups with different codes (a, b) in a column ($p < 0.05$).

It was reported that use of high-tannin sorghum reduced weight gain and feed efficiency in poultry (Elkin *et al.* 1996). Feeding high tannin sorghum in Japanese quail, leads to reduced growth compared to low tannin sorghum (Armanious *et al.* 1973; Armstrong *et al.* 1973; Luis and Sullivan 1982; Grawood and Rogler 1987; Douglas *et al.* 1991). In the current study a significant increase observed on serum TP levels after replacing corn grain by 100% RS. It is reported TP values was significantly higher in the sorghum-based diets compared to the maize based diets (Medugu *et al.* 2010). Furthermore, it is reported that there were not significant differences on blood albumin, globulin, TP, Glu, calcium, phosphorus and uric acid levels after replacing corn by reconstituted or un-reconstituted grain sorghum in poultry (Kumar *et al.* 2007).

In the current study replacing 100% RS increased blood TP levels. Tannins are polyphenolic components and one of the secondary metabolites of plants. These components have differences in solubility, structure and molecular weight (Makkar 2003). The most important properties of tannins are mixture with

proteins and ions, but most of tannins tend to combine ionic bound with nutrients e.g. hydrophobic and proline rich proteins (ChaichiSemsari *et al.* 2011). Low to moderate concentrations of tannins significantly increased the net absorption of amino acids, sulphur and nitrogen digestibility which ultimately enhanced wool growth, ovulation rate, live weight gain, growth rate and milk production and quality in ruminants. These components are like a two head sward and their usage is does dependent in animals (Hassanpour *et al.* 2011). However higher amount of tannin in diet may be resulted in depressed feed intakes, formation of less digestible tannin-dietary protein complexes, inhibition of digestive enzymes, increased endogenous protein secretion, negative impacts on the gastrointestinal tract function and toxicity of absorbed tannin or its metabolites, and therefore decrease in blood TP (Selle *et al.* 2010). Total serum protein is usually a reflection of the protein quality and absorption process (Eggum 1970). Result of the present experiment is consistent with results of the previous researches. It seems decreased levels of tannin in RS improved protein absorption in chicks and then caused to increase TP levels in Japanese quails. Further studies may be conducted to investigate higher levels of replacement. Based on our previous researches there were no significant differences after feeding reconstituted sorghum on carcass yield and carcass components as well as edible internal organs of Japanese quails. Moreover, replacing 50% or 100% sorghum grain in place of corn grain has not adverse effect on carcass characteristics in Japanese quails (Emami *et al.* 2012b). As the blood parameters directly affected by intestinal absorption of the nutrients, the results of current study can be supported by our already published data (Emami and Maheri-Sis 2015), which indicate that replacing reconstituted sorghum had better effects than un-reconstituted sorghum on average villi length and average depth of lieberkuhn crypts of small intestine in Japanese quail. We have previously (Emami *et al.* 2012a) conclude that replacing 100% untreated sorghum grain in place of corn grain led to decrease growth performance of Japanese quails, thus, current data especially in case of Glu, TP, TG and HDL, may explain the results on performance characteristics.

According to the results in Table 2, there was no significant difference in blood urea levels in Japanese quail fed RS or URS based diets compared to control group. It is reported that diets with different levels of raw red sorghum or RS does not create an adverse effect on blood serum parameters (Kumar *et al.* 2007). Also, Serban *et al.* (1978) reported that there was no difference in blood urea in birds fed diets containing sorghum. Due to the effect of sorghum on serum TG, replacing URS sorghum with corn significantly decreased TG levels in Japanese quail. Satish and Asit (2009) reported that there was no significant difference in blood TG in broilers fed diets contain 75 and 100% sorghum replaced with corn. It seems that different results can related to variety of alternatives e.g. sorghum variety, birds strain and experimental diets. Beneficial effects of tannin as polyphenolic compound are well known. As seen in Table 2, RS sorghum increased serum HDL levels in quail. The maximum amount of HDL in the process observed in 50% RS whereas the lowest in the treatment with 100% URS sorghum instead of corn were treated. Furthermore, replacing URS or RS sorghums didn't have significant effects on LDL and Chl levels in Japanese quail at 42 days of age. It seems that low levels of tannin were able to decrease LDL oxidation in body (Scalbert *et al.* 2005). Accordingly, Kumar *et al.* (2007) supposed that effect of sorghum consumption on blood lipids levels of birds may be explained by the role of tannins as antioxidants which inhibits lipid peroxidation. However, further researches need to identify direct role of sorghum on blood lipids profile in poultry. In this study replacing whole corn diets with sorghum (100% URS) led to a significant reduction in blood glucose. Jansman (1993) suggested that differences in blood glucose level in chicks can be owing to elevated levels of the liver enzyme UDP-glucuronosyl transferase in animals received a diet containing high-tannin sorghum. In addition, Selle *et al.* (2010) stated that higher concentration of phytate in high tannin sorghum grain may reduce 'sodium pump' activity by approximately 80% in the jejunum and ileum, which was associated with numerical reductions in blood glucose levels. Kwari *et al.* (2011) reported that there were different effects on blood Glu after using different variety of sorghum which only the Morrow sorghum was able to significantly decrease blood Glu levels.

Sorghum is a drought-tolerant crop that offers much to the poultry industry as a significant feed ingredient. However, broilers in the starter phase experience sub-optimal performance when fed sorghum-based diets. This may reflect reduced nutrient digestion and absorption or altered post absorptive utilization. The preceding sections have identified a number of possible contributing factors. Delineation of their impact through research will allow the development of strategies to improve the nutrient utilization of sorghum. In this regards

recent findings extend on previous findings and current studies showed that sorghum has effect in Japanese quail. Finally, the authors recommend merit further investigation need to clarify direct role of sorghum as feedstuff in poultry production.

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