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The Use of Crushed Caraway (*Carum carvi*) and Black Seed (*Nigella sativa*) Additives on Growth Performance, Antioxidant Status, Serum Components and Physiological Responses of Sanjabi Lambs

Research Article

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ABSTRACT

The aim of the present study was to investigate the effect of caraway and black seed supplementation on the physiological responses, antioxidant, serum, and hematological parameters of Sanjabi lambs. 18 Sanjabi male lambs were randomly assigned to three dietary treatments: (i) basal diet (control); (ii) basal diet with added 30 grams black seed/kg dry matter (DM); and (iii) with added 30 grams caraway/kg DM. The lambs fed caraway diet had higher final body weight than that of the control ($P<0.05$). Lambs fed black seed diet had lower alanine aminotransferase and cholesterol concentrations than those of the controls ($P<0.05$). Lambs fed caraway diet had lower blood malondialdehyde compared with the control ($P<0.05$). In conclusion, caraway and black seed supplements could improve growth performance and antioxidant parameters in fattening lambs.

KEY WORDS antioxidant, malondialdehyde, performance, thyroxin, triiodothyronine.

INTRODUCTION

Modern animal production requires the use of safe and effective additives with anti-virus, anti-parasite, anti-stress, aiding digestion, improving fecundity and enhancing appetitto properties to stimulate production efficiency. Therefore, attempt to use natural materials such as medicinal plants are widely accepted as feed additives (Aboul-Fotouh *et al*. 2000). The use of medicinal plants as a natural feed additive in ruminants' diets is becoming useful rather than antibiotics or chemicals, which may have the unique advantages and an accumulative effect on human health (Xiang and Zhou, 2000). Herbs contain a variety of active ingredients, and other unknown substances promoting growth (Xiang and Zhou, 2000). They can be used as an attractant to increase feed intake, improve feed utilization and increase weight gain (Xiang and Zhou, 2000). Herbs are more compatible with body because of their normal nature and having medicine homologues components together, therefore they are most suitable, especially in cases of long consumption as well as in chronic diseases (Borimnejad, 2008). Among these species, caraway (*Carum carvi*) is one of the most appreciate spices for its seed richness, plethora of biologically active compounds and medicinal agent (Laribi *et al*. 2010). Caraway, is belongs to order Apiales, family Apiaceae, genus *Carum*, species *carvi* and it is native of western Asia, Europe, and North Africa (Ahmad and Abdel-Tawwab, 2011). Wichtl (1994) reported that caraway promotes gastric secretion, stimulates appetite, and is used as a remedy for very disease for example colic, loss of appetite, and digestive disorders. Caraway seed is used in meat, food and distillery industries due to its pleasant flavor

and intense taste (Sedlakova *et al*. 2001). Black seed *(Nigella sativa)* is an annual erect herbous plant that belongs to the family Ranunculacae or Batter-cup family (Hassan, 2015). Black seed as a remedy for cough, bronchitis, headache, rheumatism, fever, digestive disorders and allergies (Burits and Bucar, 2000) and has antitumor, antihistaminic, anti-diabetic, anti-inflammatory and antimicrobial activities (Ghosheh *et al*. 1999). Khattab *et al*. (2011) concluded that the addition of black seed oil to rations of pregnant buffalos and their offspring had positive effect on nutrient digestibility, increased immune responses and productive performance of the obtained calves. The pharmacological action of active plant substances or herbal extracts in humans is well known, but in ruminants nutrition the number of precise experiments is relatively low. Because of these properties of caraway and black seed on the health status, physiological responses, the aim of the present study was to examine the effect of dietary crushed black seed and caraway on growth performance and the antioxidant defense system in Sanjabi lambs.

MATERIALS AND METHODS

This study was carried out at the Research Station of Department of Animal Science, Razi University of Kermanshah, Iran. Animals were cared for according to the guidelines of the Iranian Council of Animal Care and the local Ethics Committee of Razi University (code no:395-1-025). Eighteen fat-tailed Sanjabi lambs (30±0.4 kg) were randomly assigned to three experimental groups in a completely randomized design. After two weeks of adaptation to a basal diet, the lambs were randomly allocated to one of the three dietary treatments: 1) basal diet as control; 2) basal diet plus 30 g/kg DM black seed; and 3) basal diet plus 30 g/kg DM caraway.

The basal diet was formulated using NRC (2007); its ingredients and chemical composition of black seed and caraway are shown in Tables 1 and 2, respectively. The experimental period lasted for 8 weeks during summer season. The lambs were individually penned (1.5×1 m pens) with free access to fresh water. Feed intake was recorded daily, by subtracting feed offered from feed refusals, at 08.00 a.m. After 16.00 hours fasting, the body weight (BW) of lambs was measured weekly throughout the experiment. Blood samples were collected via jugular puncture in the commencement and end experiment. Blood samples were placed on ice at once after collection and centrifuged at 3500 × *g* for 15 min at 4 ˚C for serum harvesting. The serum was maintained at -20 ˚C until analysis.

Blood metabolites

Albumin and total protein (TP) of serum were measured by biuret and bromocresol green dye binding method (McGinlay and Payne, 1988) and serum globulin was calculated by subtracting the values of serum albumin from the values of TP and serum glucose was measured by using specific kits and associated procedures (Pars Azmoon Co., Tehran, Iran).

The concentration of serum cholesterol was estimated by cholesterol oxidase/peroxidase and the activity of aspartate aminotransferase (AST), lactic dehydrogenase (LDH), and alanine aminotransferase (ALT) were determined. All mentioned metabolites were measured by auto-analyzing spectrophotometer (BT-3500, Spain) using specific kits and associated procedures (Pars Azmoon Co., Tehran, Iran).

Thyroxin (T4) and triiodothyronine (T3) were measured by a triiodothyronine and thyroxin ELISA kit (Diametra, Italy) and cortisol (Cortisol ELISA kit, Moonblind Inc., Lake Forest CA, USA) using the ELISA method.

Malondialdehyde measurement

The concentration of malondialdehyde (MDA) of serum was determined as thiobarbituric acid reactive substances according to Placer (1966). In brief, 0.2 mL of serum was added to 1.3 mL of 0.2 mol/L tris and 0.16 mol/L KCl buffer (pH 7.4). Then, 1.5 mL thiobarbituric acid (Sigma, USA) was added and the mixture was heated in a boiling water bath for 10 min.

After cooling, 3 mL of pyridine – butanol (3:1, v/v) (Sigma, USA) and 1 mL of 1 mol/L NaOH were added. A blank was run simultaneously by incorporating 0.2 mL distilled water instead of the serum. The absorbance of the test sample was read at 548 nm. The plasma MDA concentration was calculated by using 1.56 × 105 as the extinction coefficient.

Antioxidant capacity

Total plasma antioxidant capacity was determined by ferric-reducing antioxidant power (FRAP) assay according to Benzie and Strain (1999). The FRAP value depends upon the reduction of a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex to the ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by sample antioxidants at low pH. Fe^{2+}-TPTZ has an intensive blue color and was monitored at 593 nm.

Statistical analyses

All data analyses were performed using the general linear models (GLM) procedure of SPSS (2007) version 16. Repeated variables (blood samples) were analyzed using the repeated measure procedure by PROC MIXED. A one-way analysis of variance (ANOVA) was used to analyze the data and the means were separated by Duncan's multiple range test. Results were considered statistically significant when $P < 0.05$.

Table 1 Components, ingredients and nutrient composition of basal diet

Ingredients	Amount
Alfalfa hay	40
Concentrate	60
Concentrate ingredient (DM g/kg)	
Barley	260
Corn	200
Soybean meal	100
Dicalcium phosphate	10
Sodium bicarbonate	7.5
Mineral-vitamin mixture[1]	10
Salt	2.5
Urea	10
Chemical analyses	
Dry matter (%)	90.82
Metabolizable energy (kcal/kg)[2]	2570
Crud protein (% DM)	16.60
Ether extract (% DM)	3.75
Ash (% DM)	7.00
Neutral detergent fiber (NDF) (% DM)	36.00
Ca (% g/kgDM)	8.50
P (% g/kgDM)	5.9

[1] Mineral and vitamin (g/kg) supplement: Se: 0.01 g (Na_2SeO_3); Mn: 2 g; Zn: 0.3 g; Co: 0.1 g; I: 0.1 g; Ca: 19.5 g; P: 9 g; Mg: 20 g; Fe: 3 g; Antioxidant: 0.4 g; vitamin A: 500000 IU; vitamin: D_3: 100000 IU and vitamin E: 0.1 g.

Table 2 Chemical composition of black seed and caraway

Item	DM (%)	CP (%)	CF (%)	EE (%)	ASH (%)	OM (%)	NDF (%)	ADF (%)
Black seed	95.49	22.23	21.47	28.65	4.17	91.64	70.23	40.32
Caraway	93.46	19.25	28.70	3.81	11.69	83.84	67.64	40.13

DM: dry matter; CP: crude protein; CF: crude fibre; EE: ether extracts; OM: organic matter; NDF: neutral detergent fiber and ADF: acid detergent fiber.

Two-way repeated measure ANOVA was performed to determine the effects of sampling time and the differences between treatments and the interaction between time and treatment.

RESULTS AND DISCUSSION

The effects of caraway and black seed supplementations on lambs' performance are shown in Table 3. Lambs fed on the caraway-supplemented diet showed higher final BW than that of the controls ($P<0.05$). The treated animals had higher total BW gain compared with lambs in the control group (Table 3). The same trend in weekly BW of the experimental lambs was observed (Figure 1).

The effect of black seed and caraway supplementations on blood metabolites in lambs are presented in Table 4. Lambs fed black seed supplemented diet had higher serum glucose concentration than lambs fed the control diet.

The black seed and caraway groups showed higher A:G ratio compared to the control animals, while animals fed caraway diet had the highest A:G ratio. Black seed and caraway treatment had no effect on total protein, albumin, globulin, urea, AST and LDH concentrations than the control group. All supplemented lambs had lower blood cholesterol concentration than the control, while animals fed black seed diet had the lowest cholesterol concentration ($P<0.05$).

ALT activity in the black seed group decreased significantly compared with the control lambs ($P<0.05$). T_3 and T_4 levels and T_3:T_4 ratios were no affected by experimental treatments.

Lambs on caraway diet had higher T_3 serum concentration than the control group. The lambs received caraway exhibited lower blood MDA than the control group ($P<0.05$). There were no significant differences among the treatments for total plasma antioxidant capacity (TAC) concentration. Beneficial effects of medicinal plants or active substances in animal nutrition may include the improvement of endogenous digestive enzyme secretion, stimulation of appetite and therefore increase feed consumption, activation of immune response and antibacterial, antiviral, antioxidant actions which may affect the physiological and chemical function of the digestive tract (Rahimi *et al.* 2011). Herbal medicinal active substances have the highest stimulatory influence on bile secretion and pancreatic enzymes activity (Kalpana *et al.* 2002). Antibacterial and fungicidal properties in caraway are important in pharmaceutical applications and also in human, animal ration and veterinary medicine (Sedlakova *et al.* 2001).

According to the obtained results in this study, feed intake was not affected by treatments, although feed intake was numerically higher in treatments supplemented with feed additives compared to the control group.

Table 3 Effects of black seed and caraway supplementations on growth and feed intake of Sanjabi lamb (least-squares means±Sd)

Treatment	Parameters					
	IBW (kg)	FBW (kg)	Total gain (kg)	Feed intake (g/d)	ADG (g)	FCR (g/g)
Control	28.30±1.09	39.98±1.03^{b}	11.68±1.11	1291±0.021	166.85±15.90	7.81±0.884
Black seed	28.43±1.09	40.86±2.86^{ab}	12.43±2.74	1324±0.064	177.61±39.23	7.76±1.84
Caraway	28.53±2.64	42.77±1.10^{a}	14.24±2.98	1338±0.094	203.47±42.69	6.87±1.65
P-value	0.974	0.057	0.205	0.490	0.105	0.497

IBW: Initial body weight; FBW: final body weight; ADG: average daily gain; FCR: feed conversion ratio.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).

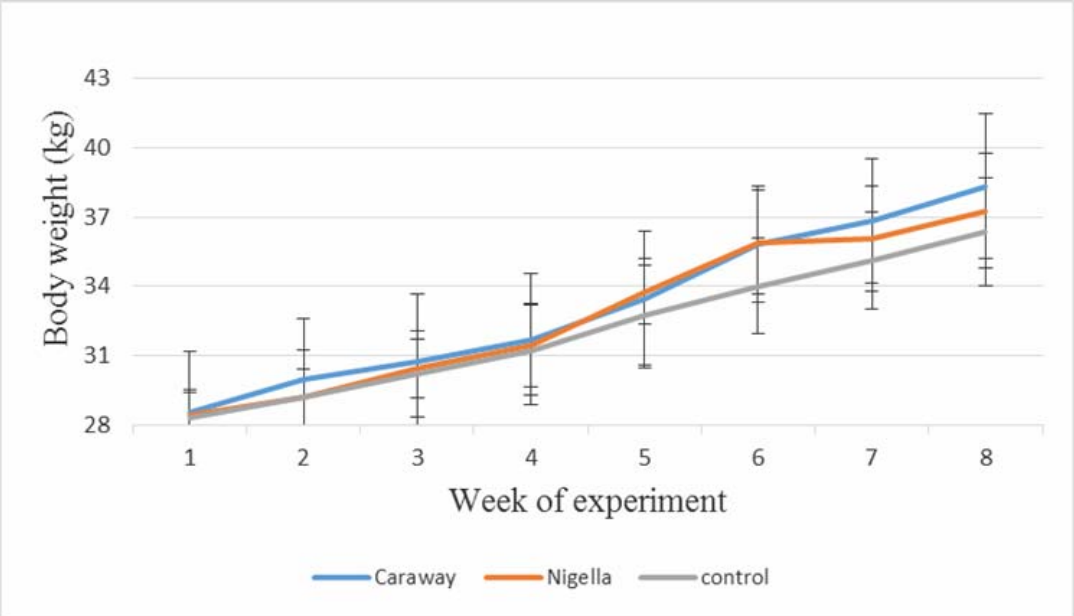

Figure 1 The same trend in weekly body weight (BW) of the experimental lambs

Table 4 Effects of black seed and caraway supplementations on blood parameters and antioxidant statue of Sanjabi lamb

Blood parameter	Experimental group			SEM	P-value
	Control	black seed	caraway		
Glucose (mg/dL)	72.16	73.14	70.40	0.997	0.551
Urea (mg/dL)	39.00	39.00	38.58	0.864	0.977
Total protein (g/dL)	8.00	7.80	7.70	0.077	0.294
Albumin (g/dL)	3.23	3.26	3.28	0.221	0.880
Globulin (g/dL)	4.76	4.53	4.42	0.090	0.300
A:G ratio	0.681	0.725	0.750	0.099	0.384
Cholesterol (mg/dL)	50.00^{a}	37.01^{b}	45.12^{ab}	0.041	0.025
AST (IU/L)	92.00	95.90	98.20	2.53	0.629
ALT (IU/L)	19.80^{a}	11.05^{b}	17.00^{ab}	0.032	0.094
LDH (IU/L)	596.33	660.00	620.83	64.64	0.597
T3 (ng/dL)	1.28	1.27	1.30	0.065	0.966
T4 (ng/dL)	8.38	8.63	8.69	0.085	0.290
T3:T4 ratio	0.153	0.147	0.149	0.301	0.883
MDA (nanomol/mL)	1.36^{a}	1.33^{a}	1.16^{b}	0.009	0.020
TAC (micromol/mL)	0.750	0.740	0.736	0.818	0.968

A:G: albumin to globulin ratio; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase; T3: triiodothyronine; T4: thyroxin; MDA: malondialdehyde and TAC: total plasma antioxidant capacity.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The positive effects on feed intake might be explained by an effect on the hypothalamus resulting in stimulation of hunger centers in the brain, stimulation of appetite and increasing the desire to eat (Petit *et al.* 1993).

Other studies confirmed such plants seeds had favorable effects on nutrient digestibility, live weight and feed efficiency with cows (Mohamed and El-Saidy, 2004; Aboul-Fotouh *et al.* 2000; Ghosh *et al.* 2011; El-Saadany *et al.*

2001). Lambs fed ration supplemented with black seed showed no significant difference on final BW and average daily gain compared to the control group. In contract, El-Rahman *et al*. (2011) indicated that Demeshgi goats fed 20% black seed showed significantly higher average daily gain compared to the control diet. Mohamed and El-Saidy, (2004) found that supplementation of black seed on camel rations improved animal performance compared to the control animals. Addition of black seed in feed increased bile flow rate, which then aid in fat digestion and absorption of fat soluble vitamins (Lewis, 1980). In agreement with this study, Mahmoud and Bendary (2014) reported that lambs fed ration supplemented with black seed showed no significant difference in term of final BW and average daily gain. Therefore, the favorable effects of black seed and caraway in this study on performance are due to their high nutritive value as well as pharmacologically active substances present in the seeds.

There are a few studies on the effects of black seed and caraway on blood metabolites of livestock in lambs. Levels of blood metabolites are an indicative of body condition (Zanouny *et al*. 2013). Glucose is the most important carbohydrate in ruminant animal biochemistry. Black seed were known to reduce blood glucose level in different animal species (Haddad *et al*. 2003). In this study, lambs fed black seed and caraway supplemented diets had no change on blood glucose concentration than lambs fed the control diet. Contrary to the results of this experiment, Zanouny *et al*. (2013) investigated the effect of supplementing black seed on male lamb performance. They concluded that the supplementation of black seed increased serum total protein, glucose, albumin, globulin, and testosterone concentrations while decreased triglycerides and cholesterol. In agreement with this result, adding black seed oil in calve diet showed no significant differences in term of plasma total protein, albumin, urea, creatin, total lipids, glucose, and other parameters (Khodary *et al*. 1997).

In contrast with our results, Khodary *et al*. (1997) indicated plasma cholesterol was significantly higher for calves fed black seed oil compared to unsupplemented group. In agreement with of our results, feeding crushed and non-crushed black seed at 3% reduced plasma cholesterol, triglycerides concentrations (Al-Beitawi *et al*. 2009). The reduction in the cholesterol level might be due to the active ingredients such as thymoquinone and compounds like mono unsaturated fatty acids that lower the cholesterol synthesis by hepatocytes and decrease the fractional absorption of cholesterol from small intestine (Brunton, 1996). MDA concentration is widely used as an indicator of lipid peroxidation (Mousaie *et al*. 2014). Caraway seed exhibited high antioxidant activity which has been attributed largely to the presence of monoterpene alcohols, linalool, carvacrol, carvone, flavonoids and other polyphenolic compounds (Najda *et al*. 2008).

CONCLUSION

Caraway supplementation increased feed intake and final BW in lambs. Lambs fed black seed and caraway supplements had lower feed conversion ratio (FCR) than lambs on the control diet. The supplementation of black seed decreased serum cholesterol and ALT concentrations than those of the controls. This study suggests that black seed and caraway supplementation are effective methods for improving the performance and metabolic status of lambs.

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