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Replacement of cereal grains with dried citrus pulp in crossbred Zell-Afshari fattening male lambs: effects on growth performance, internal organs, meat oxidative stability, and blood metabolites

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Abstract The use of low-cost alternative feed sources, such as citrus pulp, in animal husbandry may result in improved performance and animal production. This study aimed to investigate the effect of different levels of dried citrus pulp (DCP) on productive performance, carcass, and meat quality and blood biochemical parameters in fattening male lambs. Sixteen crossbred Zell-Afshari lambs (28±1.0 kg body weight) were randomly assigned to one of four groups in a completely randomized design for 75 days. The dietary treatments were a control diet and a control diet supplemented with 10, 20, or 30% DCP. Lambs fed the 30% DCP diet had increased feed intake and also had higher FCR than the control groups ($P < 0.05$), while FCR was similar in the other treatment groups ($P < 0.05$). In terms of carcass traits, the inclusion of DCP in the diets of the lambs resulted in a decrease in the weight of abdominal fat compared to the control group ($P <0.05$). Compared to the control group, lambs receiving 10, 20, or 30% DCP in their diets had a linear decrease in blood peroxide and thiobarbituric acid (TBA) indices, while their meat ash concentration had a linear increase ($P <0.05$). In contrast, the inclusion of DCP in the diets of lambs had no significant effects on serum biochemistry and liver enzyme activity. In conclusion, replacing cereal grains with 10% or 20% DCP demonstrated beneficial effects on some carcass traits and meat oxidative stability in Zell-Afshari fattening male lambs.

Introduction

In recent years, many citrus juice factories have been established in the northern regions of Iran. As a result, the by-products generated by this industry have increased, leading to various challenges for the industry owners and the surrounding environment [1]. A prominent by-product is citrus pulp, which is derived from the citrus juice industry and consists of a mixture of citrus peel, pulp and seeds [2]. In addition to its palatability, the chemical composition of citrus pulp is suitable for ruminants in terms of nutritional value. Pectin (20-25%) and crude fiber (20-23%) are the main components of citrus pulp, indicating the relatively high energy content of this by-product in ruminant diets [3]. These authors reported that citrus pulp contains approximately 6-9% crude protein and 2.3-2.7 (Mcal.kg^{-1}) of metabolizable energy. However, concerns about the adverse effects of synthetic antioxidants, such as mutagenicity, toxicity, and carcinogenicity, have prompted the search for natural alternatives [4]. On the other hand, high doses of synthetic antioxidants can cause DNA damage and induce premature senescence [4]. In recent years, considerable attention has been paid to the waste products from citrus juice factories, which contain natural antioxidants. It is well documented that citrus pulp is rich in antioxidant compounds, including flavonoids, ascorbic acid and carotenoids [5, 6, 7]. Therefore, it is hypothesized that inclusion of dried citrus pulp (DCP) in the diet of growing lambs may improve the oxidative capacity of meat after storage.

The widespread use of cereal grains such as barley or corn in diets not only increases production costs, but also contributes to digestive disorders, including acidosis or bloat in feedlot lambs [8]. Several reports have demonstrated the feasibility of substituting citrus pulp for cereal grains in the diets of fattening lambs or dairy cows without compromising productivity [9, 10]. However, little information is available on the effect of DCP inclusion on meat oxidative stability and blood biochemical parameters in finishing lambs. Therefore, the aim of this experiment was to evaluate the influence of DCP as an alternative to cereal grains in the diets on growth performance, carcass composition, blood biochemical parameters and meat quality in Zell-Afshari fattening male lambs.

Materials and Methods

Animals and experimental diets

This experiment was conducted in a commercial farm (Amol, Mazandaran Province, Iran) and all protocols were approved by the animal welfare commissioner of the Department of Animal Sciences, Faculty of Agriculture, Qaemshahr branch, Islamic Azad University, Qaemshar, Iran.

The DCP, obtained from an extraction and syrup factory, was obtained from oranges and subjected to sun drying. The product was then stored in a warehouse protected from light and moisture. The chemical composition of DCP, including dry matter, crude protein, ether extract, ash and total fiber, was determined in the laboratory using AOAC (2005) methods (Table 1).

Sixteen Zell-Afshari fattening male lambs (approximately 5 months old) with an average BW of 28±1.0 kg were randomly assigned to four treatments with four replicates in a completely randomized design. Dietary treatments consisted of a control diet and the control diet supplemented with three levels of DCP (100, 200, or 300 g/kg). All experimental diets were formulated to meet the recommendations of the National Research Council (NRC, 2007), and the ingredients and chemical composition of the diets are listed in Table 2. Metabolic cages measuring 110×105 cm with separate feed and water troughs were used in the study. The lambs were initially weighed, vaccinated against deworming and enterotoxemia, and then fed twice daily (08:00 and 16:00) with a fully mixed diet, while water was provided ad libitum. The experimental diets were fed to the lambs for 75 days.

Growth performance

To determine BW gain, individual lamb weights were recorded at the end of the experiment along with feed intake. The FCR was then calculated from the feed intake and BW gain data for each lamb [11].

Blood metabolites

Blood samples were collected from all lambs via the jugular vein after 12 to 14 hours of food restriction. Blood samples from each animal were collected in 2 separate test tubes and centrifuged (3000 g) for 15 minutes to separate the serum. Sera samples were stored at -20°C until blood biochemical metabolites were measured. Blood biochemical metabolites, including triglycerides, total cholesterol, glucose, total protein, albumin, high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), and liver enzyme activity (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using Pars Azmoon kits on an automated analyzer (Technicon RA 1000; Bayer Co., NY, USA).

Carcass traits

At the end of the experimental period, the lambs were weighed, humanely slaughtered, and carcass characteristics evaluated. After complete exsanguination, the body was skinned, the digestive tract was gently removed, and the weights of the carcass, abdominal fat, and thigh were recorded, as well as the weights of the kidneys, liver, lungs, heart, and spleen as internal organs. All data were presented as percentage of live weight of each animal. Loin-eye muscle area, which is the exposed cross-sectional area of the longissimus dorsi muscle, was measured after the left-side carcasses were cut between the 12th and 13th ribs, exposing the transversal, and the cross-sectional area was traced with a planimeter (model KP-25, USA). The backfat thickness of the left side carcasses was measured over the deepest part of the loin-eye muscle [12].

Meat quality

A portion of the thigh meat was homogenized and sent to the laboratory for evaluation of chemical composition and oxidative stability [13]. Protein, dry matter, ash, and ether extract in the thigh meat were measured according to AOAC [14] procedures. In addition, meat quality indices, including peroxide value, TBA number, pH, and malonaldehyde (MDA) content, were determined using specific methods described in the study [13,15]. The extent of lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS), expressed as grams of MDA per kilogram of meat, using the procedure described by Sante et al. (2008) [13]. Briefly, 20 g of thigh meat was mixed with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 min. The contents of the blender were raised with 50 ml of water, mixed and filtered through a Whatman filter. This filtrate is called the TCA extract and is used in the TBA evaluation. 5 ml of the TCA extract was mixed with 5 ml of 0.01 M 2-TBA. This solution was kept at room temperature for 14 hours. The absorbance at 532 nm is reported as the TBA number. However, the pH and peroxide value (PV) of the meat samples were determined according to the methods described by Aksu (2007) [15].

Statistical analysis

Data were analyzed according to the completely randomized design using one-way analysis of variance in the GLM procedure of SAS. The statistical models were:

$$Y_{ij} = \mu + A_i + e_{ij}$$

Where: Y_{ij} = the dependent variables, μ= the overall mean, A_i= the treatment effect (i = 1–4), and e_{ij}= the residual error. Data on growth performance were analyzed using the following model: $Y_{ij} = T_i + ß_i(X_{ij} - \bar{X}) + e_{ij}$

Where Y_{ij} is the observation parameters, T_i is the fixed effect of treatment on the assessed parameters, $ß_i$ is the regression coefficient, X_{ij} is the initial BW with mean $\bar{X}$ (covariate) and e_{ij} is the standard error of the term. The initial BW was used as covariate for analysis of body weight gain data.

Statistically significant differences between treatments were determined using the Tukey test at $P<0.05$. Orthogonal polynomial contrasts were also used to examine linear and quadratic trends in response to increasing levels of DCP.

Results

Figures 1, 2, and 3 show the results of growth performance indices, including feed intake, BW gain, and feed conversion ratio (FCR) of the lambs in response to different levels of DCP in their diets. Compared to the control group, lambs fed a 30% DCP diet had increased feed intake ($P < 0.05$), while the other groups had the same amount of feed intake. Dietary treatments had no significant effect on lamb BW gain. Lambs receiving 30% DCP had a higher FCR than controls ($P < 0.05$), while the other treatment groups had similar FCRs.

Except for abdominal fat, other carcass indices were not affected by the dietary treatments (Table 3). The relative weight of abdominal fat decreased in lambs fed diets supplemented with 10, 20, or 30% DCP compared with the control group (P < 0.05). As shown in Figure 4, rectus muscle surface area was similar in the different groups of lambs. The first group was fed a control diet supplemented with 10% and 20% DCP, while the second group was fed a diet supplemented with 10% and 30% DCP as well as the control group.

Table 1. Chemical compositions (analyzed) for dried citrus pulp[1]

Items (%)	Dried citrus pulp
Dry matter	86.75
Crude protein	5.94
Ether extract	0.67
Ash	9.84
Total fiber	29.29
NFC[2]	61.34

[1] Data obtained from the mean of duplicate sample
[2] Non-fiber carbohydrate = 100 − (%NDF + %CP + %Fat + %Ash)

The results of the chemical composition of the lamb meat are presented in Table 4. The concentration of ash in lamb meat did not differ significantly among lambs fed 10%, 20% and 30% DCP diets. However, the ash concentration was similar in lambs fed 10% and 20% DCP diets and in the control group. Interestingly, the addition of 30% DCP to the diet decreased the amount of ether extract in the meat compared to the other groups (P < 0.05).

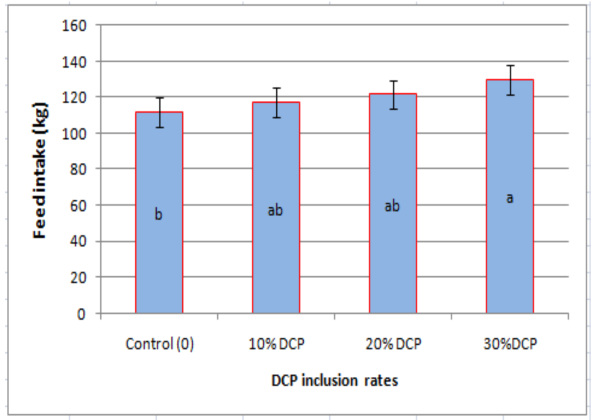

Fig 1. Effects of different levels of dried citrus pulp (DCP) in the diet on feed conversion ratio in Zell-Afshari fattening male lambs.

Values presented are means with standard error bars.
The diets were a control diet and a control diet supplemented with 10, 20, or 30% DCP.
[a,b] Indicate differences between means (P<0.05).The p-values for linear and quadratic trends were 0.03 and 0.02, respectively.

The results of the oxidative stability of the meat in response to the dietary treatments are shown in Figure 5. A linear decrease in peroxide value and MDA content was observed in meat in response to the inclusion of different levels of DCP in the diet (P < 0.05).

The results of blood biochemical parameters and liver enzyme activity are summarized in Table 5. No differences were observed in any of the blood parameters of the lambs in response to the experimental diets.

Table 2. Ingredients and chemical compositions of basal diets (as-fed basis)

		Dried citrus pulp (%)		
Indices	**Control**	**10**	20	30
Alfalfa	20.95	21.92	22.73	23.25
Dried citrus pulp	0	10	20	30
Wheat straw	20	19.22	17.53	15.07
Barley grain	31	24	16.75	10.62
Corn grain	20	18	16	14
Soybean meal	6.31	5	5	5
Dicalcium phosphate	0.29	0.44	0.59	0.72
Common salt	0.36	0.36	0.36	0.36
Calcium carbonate	1.16	1.13	1.04	0.98
Chemical composition	-	-	-	-
Crude protein (%)	15	15	15	15
Calcium (%)	0.80	0.80	0.80	0.80
Phosphorous (%)	0.40	0.40	0.40	0.40
Sodium (%)	0.18	0.18	0.18	0.18
Ether extract	4.87	4.30	4.54	4.49
Neutral detergent fiber	42.13	39.89	37.15	35.92
Metabolizable energy (Mcal/kg)	2.50	2.50	2.50	2.50

Table 3. Effects of different levels of dried citrus pulp in the diets on growth performance and carcass characteristics of Zell-Afshari fattening male lambs.

Item (kg)	DCP[1] inclusion rate					P-value		
	Control	10	20	30	SEM[2]	ANOVA	Linear	Quadratic
Body weight gain	19.53	18.78	20.53	18.98	2.04	0.45	0.27	0.09
Feed intake	111.93[a]	116.98[ab]	121.49[ab]	129.66[a]	8.11	0.02	0.01	0.18
Feed conversion ratio	5.73[b]	6.22[ab]	5.90[b]	6.83[a]	1.04	0.04	0.03	0.02
Thigh	6.21	6.26	6.24	5.97	0.27	0.87	0.94	0.87
Abdominal fat	1.00[a]	0.64[b]	0.57[b]	0.70[b]	0.08	0.02	0.04	0.01
Kidneys	0.21	0.21	0.19	0.20	0.01	0.77	0.57	0.99
Liver	0.81	0.77	0.78	0.74	0.05	0.84	0.61	0.89
Lungs	0.45	0.40	0.43	0.51	0.03	0.31	0.20	0.31
Heart	0.23	0.19	0.21	0.20	0.00	0.11	0.12	0.06
Spleen	0.10	0.10	0.11	0.10	0.00	0.42	0.91	0.37
Thickness of back fat (mm)	9.87	9.87	8.55	8.02	0.58	0.10	0.06	0.13
Area of the rectus muscle (cm^2)	35.53[b]	37.02[ab]	40.69[a]	35.57[b]	1.98	0.03	0.02	0.01

[1]Dried citrus pulp, Diets were a control diet and control diet supplemented with 10, 20 or 30% of DCP.
[2] standard errors of mean
[ab] Means within the same row with no common superscripts differ (P < 0.05).

Table 4. Effects of different levels of dried citrus pulp in the diets on chemical composition and oxidative stability of thigh meat of Zell-Afshari fattening male lambs.

Item (%)	DCP[1] inclusion rate					P-value		
	Control	10	20	30	SEM[2]	ANOVA	Linear	Quadratic
pH	5.57	5.60	5.59	5.61	0.10	0.99	0.91	0.68
Dry matter	46.83	52.19	49.64	51.85	2.94	0.56	0.99	0.43
Crude protein	14.04	14.03	14.00	13.85	0.45	0.98	0.89	0.52
Total ash	0.54[b]	0.62[ab]	0.71[a]	0.63[ab]	0.04	0.02	0.04	0.03
Ether extract	19.14[a]	19.10[a]	18.74[a]	14.87[b]	3.25	0.03	0.4	0.01
Peroxide value (mg/kg)	5.00[a]	3.37[b]	3.35[b]	1.75[c]	0.50	0.01	0.02	0.06
TBARS[3] (mg MDA[4]/kg)	5.53[a]	4.73[ab]	3.96[b]	3.89[b]	0.31	0.02	0.01	0.03

[1]Dried citrus pulp, Diets were a control diet and control diet supplemented with 10, 20 or 30% of DCP.
[2] Standard errors of mean
[3] Thiobarbituric acid reactive substances
[4] Malondialdehyde
[abc] Means within the same row with no common superscripts differ (P < 0.05).

Discussion

The mixed results observed in the present experiment regarding feed efficiency in response to citrus pulp supplementation are consistent with the mixed results reported in previous studies. No effect on feed efficiency has been reported in fattening mutton goats fed diets supplemented with citrus pulp [16], while a positive effect on FCR was observed in fattening goats [17]. The feed intake results are in contrast to the previous findings [18], which indicated that the inclusion of citrus pulp in the diet of Dalagh lambs had no effect on feed intake. In parallel with our results, a significant increase in the consumption of protein, organic matter and dry matter was observed in fattening lambs due to the inclusion of orange pulp in the diet [19]. It has been reported that the increase in orange pulp resulted in higher dry matter and protein consumption, improved protein digestibility and consequently increased nitrogen uptake [20]. They attributed these effects to enhanced rumen fermentation facilitated by the presence of pectin. According to these authors, pectin, as a rapidly and extensively fermented component of the cell wall, contributes to the rapid breakdown of the cell wall of DCP, potentially resulting in increased dry matter intake.

Table 5. Effects of different levels of dried citrus pulp in the diets on blood biochemical parameters and liver enzymes activity of Zell-Afshari fattening male lambs.

Item	DCP[1] inclusion rate					P-value		
	Control	10	20	30	*SEM*[2]	*ANOVA*	*Linear*	*Quadratic*
Glucose (mg/dl)	63.25	62.75	67.00	64.50	1.37	0.18	0.79	0.23
Cholesterol (mg/dl)	60.50	57.25	50.75	55.75	5.33	0.64	0.56	0.99
Triglycerides (mg/dl)	37.25	36.50	39.00	39.00	3.60	0.94	0.65	0.61
HDL[3] (mg/dl)	29.50	28.75	28.00	28.25	1.96	0.95	0.87	0.55
LDL[4] (mg/dl)	34.00	32.00	26.50	28.25	2.41	0.16	0.89	0.09
ALT[5] (U/L)	17.25	22.25	19.50	18.75	4.11	0.85	0.76	0.85
AST[6] (U/L)	113.25	109.75	129.50	130.75	12.15	0.51	0.68	0.85
LDL /HDL	1.17	1.10	0.97	1.00	0.07	0.23	0.12	0.41
BUN[7] (mg/dl)	23.50	22.00	22.00	25.00	1.52	0.47	0.16	0.37
Total Protein (g/dl)	7.60	7.87	7.95	7.77	0.20	0.65	0.56	0.99
Albumin (g/dl)	3.07	3.10	3.15	3.10	0.09	0.95	0.87	0.60

[1]Dried citrus pulp, Diets were a control diet and control diet supplemented with 10, 20 or 30% of DCP.
[2] Standard errors of mean
[3] High-density lipoproteins
[4] Low-density lipoproteins
[5] Alanine amino-transferase
[6] Aspartate amino-transferase
[7] Blood urea nitrogen

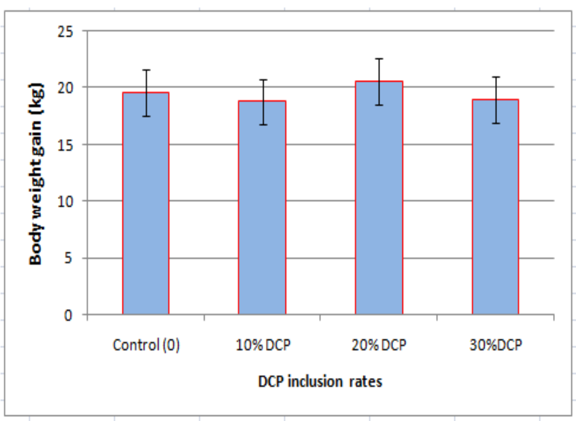

Fig 2. Effects of different inclusion rates of dried citrus pulp (DCP) in the diet on body weight gain in Zell-Afshari fattening male lambs.

Values presented are the means with standard error bars.
The diets were a control diet and control diet supplemented with 10, 20, or 30% of DCP.
The p-values for the linear and quadratic trends were 0.27 and 0.09, respectively.

Recent study results have shown that different levels of DCP are effective on abdominal fat in feedlot lambs.

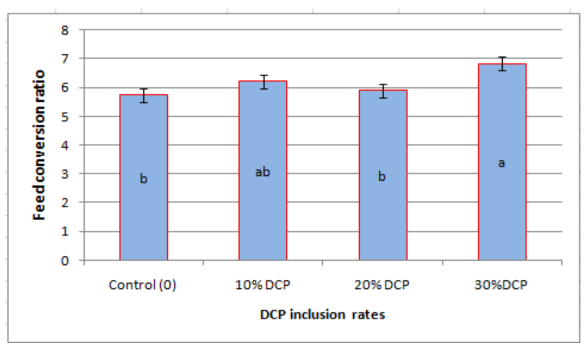

Fig 3. Effects of different levels of dried citrus pulp (DCP) in the diet on feed conversion ratio in Zell-Afshari fattening male lambs.

Values presented are means with standard error bars.
The diets were a control diet and a control diet supplemented with 10, 20, or 30% DCP.
[a,b] Indicate differences between means (P<0.05).
The p-values for linear and quadratic trends were 0.03 and 0.02, respectively.

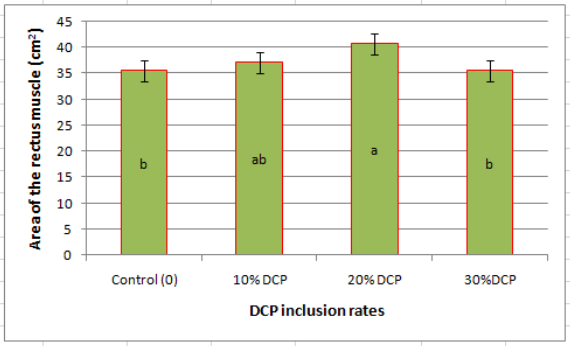

Fig 4. Effects of different inclusion rates of dried citrus pulp (DCP) in the diet on the cross-sectional area of the rectus muscle (cm^2) in Zell-Afshari fattening male lambs.

Values presented are the means with standard error bars. Diets were a control diet and control diet supplemented with 10, 20 or 30% of DCP.
^{a,b} Indicate differences between mean values (P<0.05). The p-values for the linear and quadratic trends were 0.02 and 0.01, respectively.

Data on the effects of dietary DCP on abdominal fat or rectus muscle surface area in lambs are limited. Therefore, direct comparisons cannot be made. However, it was observed that the inclusion of citrus pulp in diets had no significant effect on the percentage of abdominal fat in lambs [21]. The peroxidation-induced destruction of meat fats and proteins leads to the deterioration of its odor, flavor, texture and color during storage, commonly referred to as rancidity. Therefore, incorporating natural antioxidants into livestock diets delays meat oxidation and thereby improves meat quality attributes. In the present experiment, diets supplemented with DCP improved the oxidative stability of meat in lambs, which is supported by the results of the [21] who demonstrated that the addition of fresh citrus pulp had a positive effect on lipid oxidation, resulting in lower MDA levels in lamb meat. Consistent with these findings, a similar trend was observed in animals fed diets with different levels of DCP [22]. The preventive effect of the DCP diets against lipid oxidation observed in our experiment was probably due to the presence of high levels of antioxidant compounds such as flavonoids in DCP [23]. These polyphenolic compounds are secondary metabolites of plants with different properties, especially based on their antioxidant or antimicrobial activity [24]. Several studies have been conducted on the antioxidant properties of citrus by-products. Guo et al. (2018) identified antioxidant and phenolic flavonoid compounds in the non-volatile components of the methanol extract of citrus peel. In addition, phenolic compounds and ascorbic acid in citrus peel were observed to significantly inhibit lipid oxidation [26].

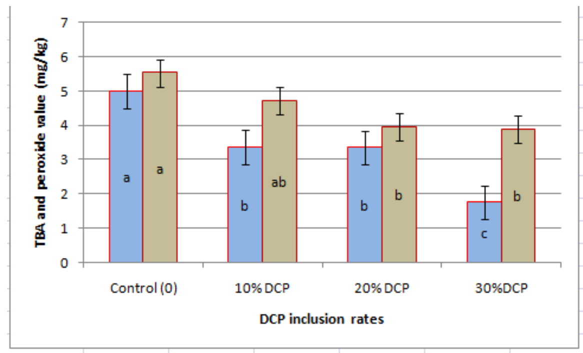

Fig 5. Effects of different levels of dried citrus pulp (DCP) in the diet on the peroxide number (blue column) and thibarbituric acid (TBA) number (gray column) in Zell-Afshari fattening male lambs.

Values presented are the means with standard error bars. Diets were a control diet and control diet supplemented with 10, 20 or 30% of DCP.
^{a,b} Indicate differences between means (P<0.05).
The p-values for the linear and quadratic trends of peroxide value were 0.02 and 0.06 and were 0.01 and 0.03 for TBA number, respectively.

There are a limited number of reports on the effects of citrus by-products on blood parameters in fattening sheep. Conflicting results have been reported in the literature. In contrast to the present findings, a decrease in serum glucose concentration in response to dietary citrus pulp has been reported in feedlot lambs [27]. According to these authors, the decrease in glucose concentration in diets containing citrus pulp may be attributed to a numerical change in propionate concentration in rumen fluid and plasma. There were no differences in blood serum concentrations of glucose, total protein, albumin, globulin, urea, triglycerides, and phospholipids in response to the inclusion of DCP in the diets of dairy cows [20]. However, the effect of dietary DCP inclusion on blood serum metabolites in dairy cows was investigated and it was reported that serum cholesterol concentrations were higher when cows were fed the diet containing DCP [28]. In the present experiment, the serum concentration of cholesterol decreased numerically in lambs fed DCP diets. The citrus pulp contains a substance called terpenes, which may affect the metabolism and biosynthesis of cholesterol in the body, preventing its increase in serum [18]. On the other hand, it has been reported that the isoflavones present in citrus pulp reduce the level of cholesterol in the liver by increasing the activity of HDL receptors and increasing LDL catabolism and, as a result, the serum concentration of cholesterol [29].

Conclusion

Based on the results of this study, BW gain was the same for all diets. When cereal grains were replaced with 10% or 20% DCP, there was no significant difference in FCR and feed intake. In addition, replacing cereal grains with 10% or 20% DCP had positive effects on some carcass traits and meat oxidative stability, including peroxide or MDA levels. Therefore, it is recommended to replace cereal grains with 10% or 20% DCP in the diet of Zell-Afshari fattening male lambs. Moreover, considering the price fluctuations of feed ingredients and especially cereal grains, the use of DCP as a suitable alternative to grains in lamb feeding may be useful in terms of more economic benefits for producers.

Conflict of interest

The authors declare that they have no competing interests.

Ethical approval

All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Acknowledgements

Not applicable

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