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SHORT COMMUNICATION

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Growth performance, nutrient digestibility and blood parameters of fattening lambs fed diet replacing corn with orange pulp

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Abstract

The objective of the present study was to evaluate the effect of replacing corn with orange pulp (OP) on growth performance, rumen fermentation, nutrient digestibility and blood parameters of fattening lambs. Twenty male lambs were placed in individual pens and fed with four levels of replacement of corn by OP (0, 33, 66, 100%) during 60 days. Average daily gain (ADG) showed a quadratic effect (p<0.007) with the increasing levels of replacement. Inclusion of 33 and 66% of OP in the diet significantly increased dry matter intake (DMI) compared to control group (p<0.01). Ruminal ammonia-N concentration showed a linear decrease (p<0.002). Ruminal fluid pH increased linearly with the increasing replacement of corn by OP (p<0.001). Acetate concentration showed a linear increase (p<0.001). Plasma total protein showed a linear increase (p<0.002). Organic matter, crude protein and neutral detergent fiber showed a quadratic effect with the level of replacement. The results of the present study showed that replacement of corn by OP improves DMI of fattening lambs, leading to an enhancement in ADG at the replacement level of 40.3%. Also, total replacement of corn by OP did not have any adverse effect on growth performance, rumen fermentation, nutrient digestibility and blood parameters.

Additional key words: average daily gain; rumen fermentation parameters; dry matter intake.

Abbreviations used: ADF (acid detergent fiber); ADG (average daily gain); CP (crude protein); DM (dry matter), DMI (dry matter intake); FCR (feed conversion ratio); NDF (neutral detergent fiber); OM (organic matter); OP (orange pulp)

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Introduction

There is a growing interest in most countries to use low cost alternative feedstuff sources for animal husbandry. One example of such feedstuff is the citrus pulp, which is a by-product originated from the citrus juice industry that includes mixture of citrus peel, pulp and seeds (Lashkari *et al.*, 2014). Large volumes of citrus fruit residue is generated yearly in Iran and also Mediterranean countries such as Spain (Bampidis & Robinson, 2006).

Fattening lambs need high levels of starchy feed to supply their energy requirements. However, the inclusion of high levels of starch decreases rumen pH (Carro *et al.*, 2000) which may affect the function of rumen cellulolytic bacteria (Stritzler *et al.*, 1998), thus decreasing rumen cell wall fermentation, digestibility and feed intake (Castrillo *et al.*, 1991). Replacement of starch with pectin appears to improve rumen function because of reduced negative associative effects (Barrios-Urdaneta *et al.*, 2000). Citrus pulp has a high concentration of pectin, which leads to a fast rumen fermentation, releasing energy for a rapid microbial growth. Since pectin is a soluble fiber, it produces less lactate than starch during fermentation (Hall *et al.*, 1998) and creates better rumen conditions for fiber fermentation. Its high level of potential degradable dry matter (Silva *et al.*, 1997) provides a high total digestible nutrient content (DePeters *et al.*, 1997). The citrus pulp has been fed successfully to dairy cows (Belibasakis & Tsirgogianni, 1996) and fattening goats (Bueno *et al.*, 2002). Martínez-Pascual & Fernández-

Carmona (1980) reported that diets containing up to 30% citrus pulp can be fed to fattening lambs without any adverse affects on growth performance, but the animal response was poorer with higher feeding levels. Also, Lanza *et al*. (2015) showed that inclusion of citrus pulp in the diet (up to 35%) of growing lambs improved intramuscular fatty acid composition without affecting growth performance. However, to our knowledge, no study has evaluated the impact of replacement of corn by orange pulp on growth performance in relation to changes in nutrient digestibility, rumen fermentation and blood metabolite concentrations. Therefore, the aim of this experiment was to study the effect of replacing corn with orange pulp on growth performance, nutrient digestibility, rumen parameters and blood parameters of fattening lambs.

Material and methods

All experimental procedures were approved by the Advisory Committee of University of Tabriz Research Council. Twenty *Ghizel* male Lambs (3-3.5 months old) with an average weight of 27.3±1.3 kg were assigned to one of the four treatments according to a randomized complete design. The lambs were confined in individual metabolic cages with feces collectors (110 × 100 cm) and equipped with water and feed troughs. All lambs were weighed, identified, vaccinated against enterotoxaemia and treated against internal parasites at the beginning of the experiment. Lambs were weighed in the morning prior to feeding. Animals were weighed at 15-day intervals through the trial and average daily gain (ADG) was determined by dividing weight gain (Initial body weight – Final body weight) by the number of days. The first 15 days allowed animals to adapt to each experimental diet. Lambs were fed experimental diet *ad libitum*. Final 60 days were used for data collection. Feed conversion ratio (FCR) was calculated as ratio between daily dry matter intake (DMI) and average daily gain (ADG).

Before preparing diet, chemical compositions of feedstuffs were determined, and then diets formulated. Lambs were fed alfalfa hay chopped (44.4%) and concentrate mixture (55.7%) with four levels of replacement of corn by orange pulp (OP). The formulation of concentrate mixtures are shown in Table 1. Lambs were offered the experimental diets as totally mixed rations twice daily at 08:00 and 16:00 h in sufficient amounts to ensure 10% orts. The weights of feed offered and refused were recorded daily and feeds offered were dried at 105 °C for 24 h to determine DM intake. The diets were formulated according to the NRC (2007) guidelines. All the diets were iso-energetic and iso-nitrogeneous (Table 1).

In the last week of the experiment, feces were collected daily for 5 days from each lamb. Each fecal sample was mixed and a 10% subsample from each collection was bulked over the 5-day period. Also, the total amounts of feed offered and refused by the animals were recorded and subsamples bulked for subsequent analyses. Samples of feed ingredients, feed refusals and feces were dried in a forced-air oven (60 ºC), ground to pass a 2 mm screen and analyzed.

Table 1. Ingredients used in the concentrate mixture and nutrient composition of experimental diets

Ingredients (g/kg DM[1] concentrate)	Replacement of corn by orange pulp in experimental diets[2] (%)			
	0	33	66	100
Orange pulp	0	223.7	456.6	675.4
Corn grain	670.6	456.6	228.3	0
Barley	254.4	243.5	223.7	226.1
Soybean meal	74.8	75.1	91.3	97.9
Dicalcium phosphate	0	0	0	0.4
Nutrient composition of experimental diet				
Metabolisable energy (kcal/kg DM)	2.4	2.4	2.4	2.4
Crude protein (g/kg DM diet)	110.8	110.8	110.8	110.8
Calcium (g/kg DM diet)	3.4	4.3	5.3	6.7
Phosphorus (g/kg DM diet)	2.9	2.9	2.9	2.9
Neutral detergent fiber (g/kg DM diet)	236.7	248.1	263.0	277.7
Acid detergent fiber (g/kg DM diet)	176.3	189.1	203.4	217.5

[1] DM: dry matter. [2] Diets contained the following: vitamin A, 6,000,000 IU; vitamin D3, 1,200,000 IU; vitamin E, 5 g; vitamin K3, 1 g; vitamin B1, 1 g; vitamin B2, 2 g; vitamin B12, 8 mg; vitamin B6, 1 g; pantothenic acid, 3 g; folic acid, 250 mg; niacin amide, 5 g; biotin, 3 mg; choline chloride, 15 g; methionine, 15 g; manganese oxide, 10 g; ferrous sulfate, 4 g; potassium iodide, 60 mg; cobalt sulfate, 20 mg; copper sulfate, 3 g; zinc sulfate, 2 g; and sodium selenite, 5 g.

Samples of ruminal digesta were collected from rumen of each lamb on day 58 (3 h after daily feed delivery). A portion of the whole rumen fluid was squeezed through 4 layers of cheesecloth, and ruminal pH was determined immediately using a portable pH meter. Following pH determination, 5 mL of filtrate were preserved by adding 1 mL of meta-phosphoric acid to determine volatile fatty acid, and 10 mL of filtrate were preserved by adding HCl to determine ammonia-N. Samples were immediately frozen (−20 °C) for later analysis. Ammonia-N concentration was determined by direct distillation in Kjeldahl equipment according to Cajarville *et al*. (2006). Volatile fatty acids were quantified using gas chromatography (WCOT Fused Silica Capillary, chorompack CP 9002) flame ionization detection and crotonic acid was the internal standard.

Blood samples were collected from each animal on day 52 (3 h after the daily feed delivery) of experimental period. Samples were taken from the jugular vein using a 10 mL syringe. Blood samples were collected into sterile blood tubes and immediately placed in ice before centrifuging at 4000 × g for 15 min to obtain serum. The serum was immediately frozen at -20 ºC for subsequent biochemical analysis. Blood parameters, including glucose, total protein, urea-N, total triglycerides, total cholesterol, high density lipoprotein and low density lipoprotein, were measured using enzymatic procedures and commercial kits (Pars Azmon Co., Tehran, Iran).

Feed, fecal and refusal samples were analyzed for their dry matter, crude protein (CP), ash content and ether extract (AOAC, 2005). The concentration of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was determined as described by Van Soest *et al*. (1991) with the inclusion of heatstable α-amylase and without the use of sodium sulfite.

Data with repeated-measurements (DMI and ADG) were analysed by mixed procedure using SAS Institute (2001) software. Data from digestibility and blood parameters were analysed with ANOVA, by using the general linear model procedure in SAS Institute (2001) software. Means were compared using Duncan's multiple range tests. For all analyses, the individual lamb served as the experimental unit. Treatments were considered significant at $p<0.05$. Regression curves (linear and quadratic) were fitted to describe the effect of replacement levels on the variables evaluated and were selected for the best determination coefficient (R^2) and p-value.

Results and discussion

Dry matter intake and growth performance of lambs fed different levels of OP are presented in Table 2. No significant differences were observed among the groups regarding final live-weight. The ADG showed a quadratic effect with the level of replacement, described by the equation: $Y=-0.017X^2+1.38X+194$ ($R^2=0.60$, $p<0.007$) and the maximum estimated value for replacement was 40.3%. Similarly, Bueno *et al*. (2002) found that Saanen kids fed different proportion of OP showed a quadratic effect and the highest ADG observed in kids fed diet containing 42.3% of OP. Also, our results were in agreement with those of Martínez-Pascual & Fernández-Carmona (1980) who found that partially replacement of citrus pulp in the fattening diets increased ADG. However, ADG of lambs fed 66 and 100% OP were lower than that of lambs fed 33% of OP ($p<0.01$). Bueno *et al*. (2002) demonstrated that the highest level of OP reduces absorption of Ca, P and Mg and may cause metabolic disorders when used for long periods. Higher DMI in lambs fed 33 and 66% of OP compared to control group might be due to increase in rumen out flow rate. Fonseca *et al*. (2001) reported that increasing the amount of OP in diets of fattening lambs led to increase in rumen out flow rate. Also, in agreement with our results, Ammerman *et al*. (1963) observed that the inclusion of OP in the diets increased DMI and stated that rations containing OP are more palatable than those containing corn.

Rumen fermentation characteristics are also presented in Table 2. Ruminal fluid pH increased linearly with the increasing replacement of corn by OP ($p<0.001$). The lowest pH value was observed for lambs fed with the control diet. In agreement with our findings, Piquer *et al.* (2009) reported that ruminal fluid pH increased linearly with the increasing replacement of wheat by OP and the highest ruminal fluid pH was observed in sheep fed diet with highest level of citrus pulp (39% of citrus fruits). They also reported that cereal-based diets produced ruminal pH values lower than citrus by-products diets. The lower pH values of the cereal-based diets could be due to the lactate production from degradation of starch which is not produced with pectin rich diets (Leiva *et al*., 2000). The ruminal ammonia-N concentration decreased linearly ($p<0.001$) when the OP inclusion level increased which agreed with findings of Piquer *et al.* (2009). The lowest ruminal ammonia-N concentration in lambs fed 100% OP compared to other experimental diets could be explained by the higher amount of NDF and pectins, because the ruminal bacteria fermenting fiber utilize mostly N from ammonia source (Russell *et al*., 1992; Hristov & Ropp, 2003). Also, the lower ruminal ammonia-N produced by citrus by-products diets might be related to higher fermentation rate and microbial growth (Lashkari &Taghizadeh, 2015).

Regarding the effect of OP inclusion on the ruminal volatile fatty acid profile, in the ruminal fluid of lambs

Table 2. Effects of replacing corn with orange pulp on performance, rumen parameters and nutrient digestibility of fattening lambs.

	Replacement of corn by orange pulp in experimental diets (%)						Polynomial contract	
	0	33	66	100	SEM[1]	*p*-value	Linear	Quadratic
Initial body weight (kg)	27.2	27.5	26.5	27.7	0.63	0.97	--	-
Final body weight (kg)	38.6	41.3	39.0	37.1	1.07	0.56	0.21	0.58
ADG (g/day)	194.2^b	241.2^a	203.3^b	169.8^b	20.53	0.01	0.09	0.007
DM intake (kg/day)	1.0^b	1.1^a	1.1^a	1.1^a	0.06	0.002	0.76	0.26
FCR	5.6	5.6	5.6	6.4	0.38	0.29	0.09	0.47
Rumen parameters								
pH	6.29^c	6.45^{ab}	6.50^a	6.43^b	0.21	0.001	0.001	0.07
Amonia-N (mg/L)	128.0^a	112.6^b	102.8^{bc}	99.8^c	1.45	0.002	0.002	0.10
Acetate (mmol/L)	52.2^b	60.6^a	63.4^a	62.1^a	1.45	0.03	0.01	0.21
Propionate (mmol/L)	31.2^a	25.4^b	25.5^b	26.9^b	0.69	0.003	0.001	0.10
Butyrate (mmol/L)	18.4	20.4	18.0	19.3	0.80	0.57	0.86	0.10
Iso-butyrate (mmol/L)	1.5	1.8	2.0	2.0	0.14	0.55	0.28	0.64
Valerate (mmol/L)	0.9	1.0	1.2	1.6	0.10	0.09	0.90	0.40
Nutrient digestibility (g/kg nutrient)								
DM	795.2	822.7	812.6	814.5	3.79	0.06	0.03	0.06
Organic matter	815.2^b	852.7^a	842.6^a	834.5^a	4.50	0.007	0.03	0.003
Crude protein	685.6^b	719.0^a	705.5^a	701.5^{ab}	5.05	0.001	0.02	0.008
Ether extract	577.0	604.5	594.4	596.3	3.79	0.051	0.03	0.06
NDF	576.2^b	626.2^a	637.7^a	636.7^a	7.85	0.002	0.002	0.02
ADF	581.7^b	659.0^a	679.0^a	677.0^a	10.7	0.001	0.001	0.001

ADG: average daily gain. DM: dry matter. FCR: feed conversion ratio. NDF: neutral detergent fiber. ADF: acid detergent fiber. [1] SEM: standard error of means. Means in a row with different superscripts differ ($p<0.05$).

fed the diets with OP acetate ($p<0.01$) and propionate ($p<0.001$) increased and decreased linearly, respectively. A similar trend was also reported by Piquer *et al.* (2009). In addition, the same was observed by Ben- Ghedalia *et al*. (1989) who reported an increase of acetate and a decrease of propionate in the rumen fluid of Merino lambs when citrus pulp replaced barley grain in the diet. As could be expected for starchy diets, the control diet resulted in higher propionate and lower acetate proportions than the citrus enriched diets. Our results agreed with Ben-Ghedalia *et al*. (1989) who reported an increase of acetate and a decrease of propionate in the rumen fluid of Merino lambs when dried citrus pulp replaced barley grain in the diet.

Table 2 presents in addition the apparent nutrient digestibility of lambs fed different levels of OP. Digestibility of DM and ether extract was unaffected by different levels of OP ($p>0.05$). The OM digestibility showed a quadratic effect with the level of replacement, described by the equation: $Y=-0.012X^2+1.16X+817$ ($R^2=0.61$, $p<0.003$) and the maximum estimated value for replacement was 57.0%. Probably, the combination of corn and OP which has different fermentation rates caused an improvement in OM digestibility (Lashkari & Taghizadeh, 2015). There was a quadratic effect of replacing corn with OP on the CP digestibility described by the equation $Y=-0.008X^2+0.93X+688$ ($R^2=0.52$, $p<0.008$) and the maximum estimated value for replacement was 55.1%. Also, these effects might be attributed to both highly digestible NDF and high content of nonstructural carbohydrate mainly comprised by neutral detergent soluble fiber of OP (Lashkari & Taghizadeh, 2015). In contrast to our finding, Bueno *et al*. (2002) showed that OM and CP digestibility did not response to different levels of OP. There was a linear effect of replacing corn with OP on NDF digestibility, however, ADF digestibility showed a quadratic effect with the level of replacement, described by the equation $Y=-0.018X^2+2.7X+583$ ($R^2=0.90$ $p<0.001$) and the maximum estimated value for replacement was 75.3%. The positive effect of replacing starchy concentrates by feeds rich in easily degradable cell walls such as OP has generally been associated with a more favorable rumen environment for cellulolytic bacteria; thus an

Table 3. Effects of replacing corn with orange pulp on blood parameters of fattening lambs (mg/dL)

	Replacement of corn by orange pulp in experimental diets (%)						Polynomial contract	
	0	33	66	100	SEM[1]	*p*-value	Linear	Quadratic
Blood urea-N	13.4[a]	11.4[c]	12.4[b]	12.8[b]	0.20	0.001	0.003	0.001
Glucose	61.2	62.5	63.4	62.2	0.35	0.11	0.09	0.20
Total protein	73.2[b]	77.2[a]	74.5[ab]	76.2[a]	0.55	0.03	0.01	0.20
Total cholesterol	64.7[b]	66.9[ab]	68.1[b]	69.9[a]	0.69	0.03	0.60	0.08
LDL-Cholesterol	48.9	50.2	52.3	51.2	0.78	0.65	0.51	0.47
HDL-Cholesterol	31.4	32.7	32.5	32.5	0.37	0.49	0.30	0.49
Total triglycerides	22.8	23.7	23.8	24.0	0.36	0.006	0.32	0.58

[1] SEM, standard error of means. Means in a row with different superscripts differ ($p<0.05$).

improvement in forage degradability (Barrios-Urdaneta *et al*., 2000). Increased rumen pH in lambs fed OP in the current study proved these findings (Table 2). In addition, improved NDF and ADF digestibility in the lambs fed diet containing OP in the current study might be attributed to differences in their composition of cell wall. This suggests that the fiber in OP is more digestible than that of corn grain. Similarly, it has been demonstrated that NDF digestibility increased linearly with the increasing replacement of corn by OP. Bhattacharya & Harb (1973) reported that inclusion of OP in the diets increased the fiber digestibility compared with basal diet from 34% to 65%, respectively. Likewise, improvement of the digestibility in these fractions seems to be associated with the low indigestible ADF and indigestible lignin in cell wall content of OP (Lashkari & Taghizadeh, 2013). High fermentable cell wall fractions in citrus pulp might lead to increase NDF digestion with diets containing OP.

Blood parameters including plasma urea-N, glucose, total protein, total cholesterol, LDL- cholesterol, HDL-cholesterol, total triglyceride of lambs fed different levels of OP are presented in Table 3. Replacement of corn by OP in experimental diets had no significant effect on plasma glucose concentration. This result was in agreement with that of Bhattacharya & Harb (1973) who reported that there was no significant difference in the plasma glucose of lambs fed 0, 20, 40 and 60% levels of citrus pulp. However, Oni *et al*. (2008) showed that plasma glucose concentration quadratically responded to increasing the proportion of OP in the diet. Plasma urea-N showed a quadratic decrease ($Y=-0.0005X^2+0.05X+13$, $R^2=0.71$, $p<0.001$) with the increase of replacement of corn by OP and the minimum estimated value for replacement was 56.1%. The lowest plasma urea-N in lambs fed 33% of OP compared to other control groups might be due to the better synchronization between carbohydrate and protein source. The lower plasma urea-N in lambs fed OP in our study is in line with a lower ammonia-N of rumen content in these groups (Table 2). It has been suggested that synchronization of the rate of carbohydrate degradation and nitrogen released in the rumen would increase the amount of retained nitrogen for growth and thus reduce the concentration of rumen ammonia-N (Sniffen *et al*., 1992). Total plasma protein increased linearly with the increasing replacement of corn by OP. These results are in agreement with those of Oni *et al*. (2008) who reported that total plasma protein increased linearly with the increasing levels of citrus pulp. The higher total plasma protein in lambs fed 33% of OP might reflect the higher supply of digestible protein in small intestine. This corroborate the results of Highfill *et al*. (1987) who reported that efficiency of microbial protein synthesis improved in the rumen of mature cows when citrus pulp was used as a supplement (218 g/kg of diet DM) for fescue hay compared with a similar level of corn. Our results were in line with results of Lunn & Austin (1983) who reported that adequate digestible protein was responsible for increase the plasma protein concentration. Total plasma cholesterol in lambs fed OP increased with compared to control group. In agreement with these findings, Belibasakis & Tsirgogianni (1996) reported that diets containing 20% of citrus pulp increased total plasma cholesterol. According to them, the high citrate content in the citrus pulp increases cytoplasmic citrate and might provide a substrate for cholesterol *de novo* synthesis and, possibly, some activation of acetyl-CoA carboxylase.

In summary, the results showed that OP can be used as a high energy feed in rations that support growth in fattening lambs. Replacement of corn by OP improved DMI of fattening lambs, leading to an enhancement in ADG at the replacement level of 40.3%. Also, total replacement of corn by OP did not have any detrimental effects on growth performance, nutrient digestibility and blood parameters.

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