Colegio de Postgraduados

Productive and Ruminal Microbiological Behavior of Sheep Fed with Two Levels of Dehydrated Orange Residue

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ABSTRACT

Objective: To evaluate the productive and ruminal microbiological behavior of sheep fed with two levels of dehydrated orange residue (DOR).

Design/methodology/approach: Thirty Dorper×Katahdin crossbred male sheep with an average live weight of 22±1.0 kg were distributed in a completely randomized design, with three treatments and ten repetitions. The distribution of treatments was as follows: T1=diet with 0% DOR (control), T2=diet with 15% DOR, and T3=diet with 30% DOR. The variables evaluated were daily weight gain, dry matter intake, feed conversion, ruminal pH, and microbiological analysis.

Results: There were no significant differences ($p>0.05$) in the productive variables due to the inclusion of DOR in the diet. Similarly, the concentration of ruminal microorganisms did not present significant differences between treatments.

Study limitations/implications: The study did not include an economic analysis that would demonstrate a reduction in production costs by decreasing the inclusion of maize in the diet.

Conclusions: Maize grain is one of the most commonly used cereals as an energy source in sheep feeding; however, it can be replaced by DOR up to 30% without affecting the productive and ruminal microbiological variables in fattening sheep.

Keywords: by-product, bacterium, citric, ovine

INTRODUCTION

In Mexico, the sheep inventory totals 8,766,678 heads, which are developed in both extensive and intensive systems (SIAP, 2023). In the latter, sheep are generally fattened

with grain-based diets (Nuncio-Ochoa *et al.*, 2001). In recent years, the price of grains has increased, resulting in higher production costs. Consequently, research needs to focus on finding new alternatives that partially replace the energy sources in diets for sheep feeding.

There is a wide variety of agro-industrial by-products used in ruminant feeding, such as maize cob and pine sawdust (Guerra-Medina *et al.*, 2014; Pérez-Sato *et al.*, 2020), bakery waste (Escorza-Montoya *et al.*, 2019), and agave bagasse (Guerra-Medina *et al.*, 2015). These by-products exhibit significant variability in their chemical composition, which is why it is necessary to know them before their incorporation into diets. There is a wide variety of agro-industrial by-products used in ruminant feeding, such as maize cob and pine sawdust (Guerra-Medina *et al.*, 2014; Pérez-Sato *et al.*, 2020), bakery waste (Escorza-Montoya *et al.*, 2019), and agave bagasse (Guerra-Medina *et al.*, 2015). These by-products exhibit significant variability in their chemical composition, which is the reason why a thorough understanding before their incorporation into diets is needed. Orange residue (*Citrus sinensis* L.) is a by-product of the juice industry composed mainly of peel, pulp, seeds, and a small portion of juice (Espinoza-Zamora *et al.*, 2019; Rincón *et al.*, 2005). Its chemical composition includes crude protein (10%), crude fiber (14.92%), ether extract (3.20%), nitrogen-free extract (64.51%), and ash (3.30%). Additionally, it serves as a suitable source of degradable carbohydrate fractions as an energy source (2.67 Mcal of ME), making it an excellent alternative in ruminant feeding (Bampidis & Robinson, 2006; Cabrera-Núñez *et al.*, 2020; Calsamiglia *et al.*, 2016).

Given that orange residue offers an alternative as an energy source in ruminant feeding, it is important to study whether productive and microbiological variables are unaffected by its inclusion. Therefore, the objective was to evaluate the productive and microbiological behavior of sheep fed with two levels of dehydrated orange residue, partially substituting maize grain (*Zea mays* L.) as an energy source.

MATERIALS AND METHODS

Location of the experiment

The experiment was conducted at the sheep module facilities of the Faculty of Agricultural and Livestock Sciences of the Benemérita Universidad Autónoma de Puebla, Mexico (19° 83’ 84.08” N and 97° 48’ 55.81 O), at an altitude of 1330 meters above sea level (INEGI, 2017).

Diets and Experimental Treatments

The diets (Table 1) were formulated for male sheep with an average weight of 22 ± 1.0 kg and an expected daily weight gain of 250 g d^{-1}, according to the nutritional requirements for sheep (NRC, 2007). They provided 15% crude protein and 2900 Mcal ME kg^{-1} dry matter (DM) of metabolizable energy.

The experimental treatments were as follows:

- T1 = diet with 0% dehydrated orange residue (DOR) (Control)
- T2 = diet with 15% DOR
- T3 = diet with 30% DOR

Table 1. Ingredients and composition of the experimental diets (%).

Ingredients \ Variable	Treatments		
	T1	**T2**	**T3**
Soybean meal	17.0	17.0	17.0
Urea	1.0	1.0	1.0
Crushed corn	59.0	44.0	29.0
Minerals	1.0	1.0	1.0
Molasses	5.5	5.5	5.5
Dehydrated orange residue	0.0	15.0	30.0
Alfalfa hay	15.0	15.0	15.0
Calcium carbonate	1.5	1.5	1.5

Animals, Housing, and Feeding

Thirty sheep (Dorper×Kathahdin crossbreeds) were used, with 10 animals randomly assigned to each treatment group and housed in individual pens measuring 1.2 m^2. Feed was provided ad libitum in the morning (09:00) and afternoon (16:00 h) for 60 days. A 10-day adaptation period was given, during which the animals were also dewormed and given vitamin supplements (Pérez-Sato *et al.*, 2020).

Evaluated Variables

Dry Matter Intake (DMI)

To calculate DMI, the food offered to the 30 sheep was weighed daily, and 24 hours later, the refused feed was weighed using a portable digital balance with a capacity of 3200 g and a sensitivity of 0.2 g. DMI was calculated by the difference in weight between the offered and refused food weights, divided by the 60 days of the experimental phase, and reported in g DM day^{-1} (Pérez-Sato *et al.*, 2020).

Daily Weight Gain (DWG)

Each of the 30 sheep was weighed in the morning (08:00) using a hanging digital scale with a weighing capacity of 300 kg and weighing precision of 100 g, before offering feed, both at the beginning and end of the evaluation period. Daily Weight Gain (DWG) was calculated as the difference between the final and the initial weight, reported in g animal d^{-1} (Pérez-Sato *et al.*, 2020).

Feed Conversion Ratio (FCR)

The feed conversion ratio was calculated by dividing the Dry Matter Intake (DMI) by the Daily Weight Gain (DWG) individually for each of the 30 sheep (Pérez-Sato *et al.*, 2020).

Ruminal pH

Approximately 100 mL of ruminal fluid was collected using an esophageal probe 4 hours after feeding, at days 30 and 60 of the experiment. The ruminal fluid was filtered, and pH was measured using a portable pH meter (ORION, model SA 210, USA) calibrated at two points with pH buffer solutions (4.0 and 7.0, respectively) (Ley-de Coss *et al.*, 2016).

Microbiological Analysis

The concentration of total bacteria (TB), cellulolytic bacteria (CB), and protozoa was determined at two time points (30 and 60 days after the start of the experiment). The TB concentration was determined by direct counting using a Petroff-Hausser counting chamber (Hauser Scientific, USA) on a LAUKA-HKS12 contrast microscope (total magnification 100X). The total concentration of bacteria per mL of ruminal fluid was calculated as the product of the mean number of cells counted in a volume of $0.05 \times 0.5 \times 0.2$ mm by 2×10^7 (Ley-de Coss *et al.*, 2016). The CB concentration was estimated using the most probable number (MPN) technique (Harrigan and McCance, 1966) after incubating rumen fluid in 13×100 mm culture tubes (in triplicate) containing anaerobic liquid medium prepared according to Hungate (1969) and Cobos *et al.* (2002). Positive growth was indicated by the degradation of Whatman No. 541 paper after 10 days of incubation at 38.5 °C. The concentration of protozoa per milliliter of ruminal fluid was determined using a Neubauer chamber (bright line, Marienfeld, USA).

Experimental Design and Statistical Analysis

A completely randomized design with three treatments and ten replications per treatment was employed. The results were analyzed using the general lineal model (GLM) procedure (SAS, 2006), and treatment means were compared using Tukey's test ($p \leq 0.05$).

RESULTS AND DISCUSSION

The diets were formulated aiming for a daily weight gain (DWG) of 250-300 g day^{-1} according to the nutritional requirements for intact male sheep (NRC, 2000). It can be observed from Table 2 that the DWG in the current experiment did not show significant differences and fell within the expected range regardless of the level of inclusion of dehydrated orange residue (DOR). This suggests that up to 30% DOR can be used in fattening sheep diets without significantly affecting DWG, which was also reflected in the final weight.

Lower results (238.4 and 209.2 g) were found when including 15% and 30% of fresh orange residue (FOR) in sheep diets, replacing sorghum grain (Villanueva *et al.*, 2013). This difference is likely due to higher moisture content in FOR, as increasing its quantity decreases dry matter intake, thereby affecting DWG. Similar findings were reported by Luzardo *et al.*, 2021, who noted that increasing fresh citrus pulp from 0% to 30% reduced dry matter intake but not DWG (1.580 and 1.480 kg day^{-1}). Authors such as Sharif *et al.* (2018) found no differences when including 10%, 20%, 30%, and 40% of dehydrated citrus pulp in sheep diets, concluding that up to 40% utilization is feasible without impacting productive parameters. Likewise, different inclusions (10, 20 and 30%) of dehydrated orange peel have been used in diets for goats, which increases milk fat percentage (Hernández-Meléndez *et al.*, 2015).

Regarding dry matter intake (DMI), no significant differences ($p>0.05$) were observed for any of the inclusions (15% and 30%) compared to the control treatment (0%). This indicates that the use of this by-product in sheep diets is advisable without affecting DMI. There are studies where by-products from the timber industry (such as pine sawdust) have been used as unconventional fiber sources in sheep diets, reporting a decrease in DMI, possibly due to the presence of turpentine, which is part of the resin and has a pungent taste (Pérez-Sato *et al.*, 2020).

In contrast, residues from the juice industry contain 82% total digestible nutrients and 6.7% crude protein (NRC, 2000). Additionally, they are highly palatable, which is why they are used as a source of energy to replace cereals in the ruminant feeding (Al Khawajah, 2003; Crickenberger, 1991).

Authors such as Sharif *et al.* (2018) report a DMI of 1.39 and 1.41 kg day^{-1} with the inclusion of 30% and 40%, respectively, of dehydrated citrus pulp in sheep, which are similar to the DMI observed in the present study. Similarly, Taniguchi *et al.* (1999) mentioned that they did not observe significant differences in feed intake when steers were fed diets containing different levels of dehydrated citrus pulp. Different results were obtained when 15%, 20%, 25%, and 30% fresh orange residue were included in lamb diets, where a significant decrease ($p<0.05$) in DMI was observed as the inclusion percentages increased (1.35, 1.22, 1.07, and 0.97 kg day^{-1}). These differences are again attributed to the high moisture content of the fresh residue, which limits DMI (Villanueva *et al.*, 2013).

Feed conversion ratio (FCR) (Table 2) was not significantly affected (4.60, 5.59, and 5.97) as the inclusion percentage of DOR increased (0%, 15%, and 30% for T1, T2, and T3 respectively). These results differ from those reported by Villanueva *et al.* (2013), who found significant differences in FCR (5.20, 4.80, 4.30, and 3.80) in lambs as the inclusion of fresh orange residue (FOR) increased (15%, 20%, 25%, and 30%) as a replacement for sorghum in the diet. A similar behavior was observed by Luzardo *et al.* (2021), who recorded a significant improvement in FCR (7.51, 7.37, 6.98) when increasing the inclusion of fresh citrus pulp from 0% to 30% in the diet of fattening steers. This is attributed to the fact that FOR contains fractions of highly degradable soluble carbohydrates in the rumen, which facilitates their utilization by ruminal bacteria (Espinoza-Zamora *et al.*, 2019).

Table 2. Productive variables of sheep fed with dehydrated orange residue.

Variable	Treatments			
	T1	**T2**	**T3**	**SEM**
Initial weight (kg)	23.25a†	23.25a	22.50a	3.96
Final weight (kg)	40.26a	37.96a	38.06a	2.64
Average daily gain (g d^{-1})	283.33a	245.17a	250.00a	34.92
Daily dry matter intake (g d^{-1})	1.50a	1.26a	1.41a	0.21
Feed to gain ratio	4.60a	5.59a	5.97a	0.87
Ruminal pH	6.43a	6.35a	6.12a	0.35

†=means with the same letters within each column do not differ statistically (Tukey, $p\leq0.05$). T1=diet with 15% dehydrated orange residue (DOR), T2=diet with 30% DOR, T3=diet with 0% DOR (Control), and SEM=standard error of the mean.

The consumption of easily fermentable carbohydrates increases lactic acid production, thereby decreasing ruminal pH value. However, in the present experiment, no significant differences in pH were observed across treatments (Table 2). Luzardo *et al.*, 2021 mention that inclusions of 30% of fresh citrus pulp in diets of fattening steers do not affect rumen pH (6.8) Similarly, Cruz *et al.* (2019) reported that pH remained unaffected (6.8±0.12) when dairy cows were provided with 2 kg animal^{-1} of orange peel silage.

The pH is a determinant in the fluctuations and concentrations of microorganisms in the rumen, as a low pH affects these concentrations. Since no significant differences in pH were observed with the inclusion of dehydrated orange residue (Table 2), the concentration of total and cellulolytic bacteria was not affected in the present experiment and falls within the range reported in the literature (107 to 1010 cells per milliliter of ruminal fluid) (Table 3). Additionally, the fiber present in this type of by-product is highly digestible, promoting favorable conditions for the activity of ruminal microorganisms, increasing acetic acid production, and causing moderate changes in ruminal pH value (Belibasakis and Tsirgogianni, 1996; Villarreal *et al.*, 2006). Similarly, the concentration of protozoa showed no significant differences and remained within normal concentrations.

Table 3. Concentration of total bacteria, cellulolytic bacteria, and protozoa per mL of ruminal fluid.

Period*	T1	T2	T3
Total bacteria (10^{10} bacteria mL^{-1})			
1	10.2	11.3	13.3
2	15.1	14.4	16.8
Cellulolytic (10^{7} bacteria mL^{-1})			
1	4.27	3.3	4.3
2	5.2	5.2	5.3
Protozoa (10^{4} protozoa mL^{-1})			
1	3.58	2.38	2.39
2	5.2	4.2	3.3

CONCLUSIONS

Maize grain is one of the most used cereals as an energy source in sheep feeding. However, it can be replaced by DOR up to 30% without affecting the productive and ruminal microbiological variables in fattening sheep.

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