Austral J Vet Sci **50, 95-99 (2018)** **DOI: 10.4067/s0719-81322018000200095**

SHORT COMMUNICATION

Health status and productivity of sheep fed coffee pulp during fattening

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ABSTRACT. The objective of this study was to evaluate the productivity and health of fattening lambs fed different levels of coffee pulp in their diets. Thirty-five crossbred lambs with an average weight of 21.8 kg were fed isoproteic and isoenergetic diets with different percentages of coffee pulp (T0: control; T1: 7%; T2: 14%, T3: 21% and T4: 28%), the base diet was formulated with corn, soybean meal, alfalfa stubble, molasses, urea and mineral salt. The fattening period was 98 days. Productivity was measured by weight gain, feed intake and feed conversion. At the beginning and end of the study, blood samples were taken to determine the health status using a biochemical profile and blood count. Analysis of variance was performed using the initial weight as the covariate for the productive variables and the initial values of the analytes in the blood chemistry and hematological analysis tests. The coffee pulp did not affect productivity, although the amount of neutrophils decreased ($P<0.05$) as the coffee pulp in the diet increased. In all treatments, urea exceeded the reference values, whereas creatinine was below the reference values. We concluded that the inclusion of up to 28% coffee pulp in the diet did not affect the productive parameters, decreases the neutrophil count without affect health status of lambs during fattening.

Key words: coffee, creatinine, neutrophils, ovines, urea.

INTRODUCTION

Coffee pulp is the most abundant by-product obtained in the wet process of cherry, which contains high amount of fiber and 9.8 % of crude protein (Salinas *et al* 2014). Coffee pulp has been included in ruminant feed to take advantage of this by-product and reduce feed costs. Up to 16% coffee pulp can be included when fattening sheep (Salinas-Rios *et al* 2015). In bulls, an intake of 1.65 kg of dry matter/animal/day of coffee husk does not affect weight gain; however, increasing the intake to 2.08 kg of dry matter/animal/day reduces daily weight gain (Barcelos *et al* 1997). Additionally, the digestibility decreases linearly with the inclusion of coffee husks (Souza *et al* 2006). In sheep, adding 112.5 g of coffee pulp to the diet daily during estrus synchronization and early gestation reduces the pregnancy rate, probably due to the caffeine concentration present in the by-product (Salinas-Rios *et al* 2016), which is a stimulant of the central nervous system (González and Ramirez-Mares, 2014). In addition to caffeine, coffee pulp contains natural antioxidants (Salinas *et al* 2014), which have been observed to improve the immune response (Morán *et al* 2012). Based on this finding, we consider that the inclusion of coffee pulp is viable at limited levels. However, there is little information on the maximum allowable limit that does not affect productivity parameters and sheep health. Current trends require products of animal origin to be safe, with a known origin ensuring that animals were provided with the necessary health conditions in the production system. However, this scenario is rarely the case because the fattening of sheep generates conditions of stress and susceptibility to disease (Galapero *et al* 2015). Few studies have evaluated the inclusion of different ingredients on the health statuses of sheep during fattening. Several variables measured in the blood are indicators of the health status of the animal, including cholesterol, glucose, protein, urea, creatinine, hematocrit and leukocytes (Pierre *et al* 2011, Kiran *et al* 2012, Collins *et al* 2016), for example, physiological state affects the blood metabolic profile (Sharma *et al* 2015). Coffee consumption and human health relation has been extensively studied (Lopez-Garcia *et al* 2013). Thus, the objective of this study was to evaluate the productivity and health of lambs fed different proportions of coffee pulp.

MATERIAL AND METHODS

The study was conducted in the municipality of La Trinidad Zaachila, Oaxaca, México located at a latitude 16°57' North and longitude 96°50' West at 1490 masl.

EXPERIMENTAL DESIGN AND ANIMAL MANAGEMENT

Thirty-five four-month-old Pelibuey crossbred male lambs with an average weight of 21.8 kg were used. At the beginning of the study (a week before coffee pulp suplementation), the lambs were subjected to a coproparasitoscopic examination. Based on the results, the lambs were dewormed orally with 10% Febendazole. Additionally, the lambs were vaccinated with Bobact 8 (Lab. Intervet) intramuscularly at doses of 2.5 mL.

A completely randomised design with 5 treatments of 7 lambs each was used. The lambs were housed in individual wooden pens that were 1.0 m wide x 2.40 m long x 1.20 m high with an earthen floor. The treatments were

Accepted: 15.03.2018.

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as follows: T0: control; T1: feed with 7% coffee pulp; T2: feed with 14% coffee pulp, T3: feed with 21% coffee pulp; and T4: feed with 28% coffee pulp. Due to live weight change and nutrient requirements, two diets were used. During fattening, a starter diet was given for 56 days; fourteen days were used for adaptation, and a finishing diet was provided for 42 days. The diets were isoproteic and isoenergetic and contained 89% of dry matter, 17.0% crude protein (CP), 2.7 Mcal of ME/kg of dry matter (DM) and 9.8% of crude fiber (CF) for the initiation stage and 89% of dry matter, 16.2% CP, 2.7 Mcal of ME/kg of DM and 9.8% CF for the finishing stage. The ingredients were corn, soybean meal, alfalfa stubble, molasses, urea and mineral salt. The coffee pulp was dehydrated in the sun for three days, this one had 9% of CP, 30 % of CF and 90 % of DM. Free access was provided to the feed and water.

DM, ash and CP were determined using the AOAC technique (1990). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined using the Van Soest *et al* (1991) technique.

MEASURED VARIABLES

To measure productivity, the lambs were weighed at the beginning and end of each stage. Feed and water were provided twice a day (7:00 and 16:00 h). Feed rejection was measured weekly. Feed intake was estimated using the dry matter percentage of 89%. Feed conversion ratio was calculated as feed intake divided by weight gain during fattening. At the beginning of coffee pulp supplementation and at the end of the study, 5 mL of jugular vein blood was collected in vacutainer EDTA tubes without anticoagulant (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). Then 3 mL were used for the determination of the biochemical profile, and 2 mL were used for the hemogram.

The cholesterol, glucose, protein, urea and creatinine concentrations were determined for the biochemical profile. For this purpose, the automated biochemical analyser ES-218 (Kontrol LAB) and the associated reagents, calibrator and DCL control were used. Endpoint and kinetic determinations were performed.

The haematocrit concentration and the leukocyte, neutrophil, monocyte, lymphocyte and eosinophil counts were determined by the hemogram. A Mex-Lab hematological analyser was used in capillary mode to determine the erythrocytic and leukocyte values. Due to the haematological characteristics of ruminants, the haematocrit was determined manually according to the microhaematocrit technique. A blood smear was prepared for the differential leukocyte count (Carr and Rodak 2009), stained with Wright's solution (MARK) for 5 min and then incubated with pH 7 buffer solution for 10 minutes.

At the end of the experiment, when the lambs were slaughtered, a parasite analysis was carried out.

STATISTICAL ANALYSIS

For weight gain, feed intake and feed conversion, analysis of variance was performed using the inclusion level of coffee pulp as the fixed effect and the initial live weight as the covariate. For the biochemical profile and haemogram, the initial value of each analyte was used as the covariate. The difference between means was determined by Tukey's test, with a significance value less than or equal to 0.05.

RESULTS AND DISCUSSION

All variables had a covariant effect, which is why we present adjusted means. Different alternatives have been attempted to take advantage of the large number of by-products generated in the different phases of coffee processing. Because there is a tendency to take advantage of agricultural and industrial by-products to lower production costs (Bampidis and Robinson 2006), coffee by-products have been used in animal feed (Barcelos *et al* 1997) and as a source of antioxidants during sheep fattening (Salinas *et al* 2015). In the present experiment, feed intake (1143.51 g d^{-1}), daily weight gain (167.85 g d^{-1}) and feed conversion (7.02) were not modified (P>0.05) by the inclusion of 28% coffee pulp (table 1).

Coffee pulp contains compounds such as caffeine and tannins that limit its intake (Ulloa *et al* 2003). Therefore, different treatments have been used to reduce these compounds and to increase the nutritional value of the coffee pulp. Treatments include the use of bacteria (Orozco *et al* 2008), silage and aerobic decomposition (Ulloa *et al* 2003). However, these processes could increase the cost. Therefore, the easiest method to provide coffee pulp to sheep is through dehydration and testing of different levels to determine the maximum inclusion where the productive parameters of the sheep are not reduced, and their health is not compromised. Despite these compounds being reported as antinutritional and the bitterness of the coffee pulp, the feed intake, weight gain and feed conversion were not differing among treatments. Previously, up to 16% coffee pulp was added to the sheep diet without affecting production parameters (Salinas *et al* 2015). In 201 kg heifers, a decrease in weight gain was reported when the inclusion of the coffee husk was 10.5% (Souza *et al* 2006). Likewise, a decrease in digestibility with the inclusion of coffee husks was reported.

In all treatments, the inclusion of coffee pulp in the diet of the sheep did not modify (P>0.05) the cholesterol, glucose, protein, urea and creatinine concentrations in the blood (table 2). However, the urea concentration was above the reference values reported by Núñez and Bouda (2008) for healthy sheep. Multiple metabolic factors are involved in the synthesis of urea. Therefore, one possible assumption is that the percentage of protein in the diets was above the sheep's requirements because cows given a

Table 1. Means (± standard error) of feed intake, weight gain and feed conversion of lambs fed different levels of inclusion of coffee pulp in the diet.

	Treatment				
	T0	T1	T2	T3	T4
Feed intake g d^{-1}	1098.06±65.8	1072.83±65.8	1191.70±65.8	1186.46±65.8	1168.53±65.8
Weight gain g d^{-1}	161.62±13	160.13±13	164.67±13	177.22±13	175.61±13
Feed conversion	7.04±0.23	6.72±0.23	7.45±0.23	6.73±0.23	7.18±0.23

T0: control; T1: feed with 7% coffee pulp; T2: feed with 14% coffee pulp; T3: feed with 21% coffee pulp; T4: feed with 28% coffee pulp.

Table 2. Means (± standard error) of the variables related to the biochemical profiles of lambs fed different levels of inclusion of coffee pulp in the diet.

	Treatment					
	T0	T1	T2	T3	T4	Reference values
Cholesterol	2.24±0.09	2.32±0.09	2.25±0.09	2.34±0.09	2.37±0.11	0.8-2.4 mmol/L
Glucose	4.16±0.26	4.22±0.26	3.65±0.26	3.66±0.26	4.37±0.28	3.2-4.5 mmol/L
Protein	68.27±0.93	68.66±0.93	69.05±0.93	68.88±0.93	69.96±1.0	60-75 g/L
Urea	8.37±0.31	7.59±0.31	8.29±0.31	8.30±0.31	8.53±0.33	4.0-7.0 mmol/L
Creatinine	67.11±2.5	70.91±2.5	66.82±2.5	68.16±2.5	65.12±2.9	78-118 µmol/L

T0: control; T1: feed with 7% coffee pulp; T2: feed with 14% coffee pulp; T3: feed with 21% coffee pulp; T4: feed with 28% coffee pulp. Reference values according to Núnez and Bouda 2008.

diet with a high protein have a higher urea concentration (Amanlou *et al* 2017).

Creatinine is one of the biochemical parameters used to measure muscle activity or kidney disorders. In the present study, we found that during administration of diets with or without coffee pulp, the creatinine levels were below the reference values reported by different authors (Pugh 2004, Anton and Mayayo 2007, Aceña *et al* 2008, Núñez and Bouda 2008). In studies with Jersey cattle, 12-month-old cattle had lower serum creatinine values than cattle older than 18 months of age (Gregory *et al* 2004). Therefore, the finding of creatinine values below the previously reported reference values in all treatments could be due to fattening animals having some factor that modifies this metabolite or because the reference values reported by other authors refer to animals of different ages than the crossbred animals used in the present study. Recent studies show that there is seasonal effect in the values of creatinine in sheeps decreasing in winter (Rathwa *et al* 2017).

The haematocrit concentration and the leukocyte, monocyte and lymphocyte counts in blood were not modified ($P>0.05$) by the inclusion of coffee pulp. The coffee pulp significantly reduced ($P<0.05$) the number of neutrophils in the lambs without altering the minimum reference values (table 3). Coffee pulp contains caffeine (Salinas *et al* 2014), which is widely used for its different physiological effects. For example, caffeine is used in children with apnea as an inhibitor of adenosine receptors and as a modulator of inflammatory processes to decrease the quantity of neutrophils by inhibiting IL-10 (Chavez *et al* 2011) and TNF-α expression (Horrigan *et al* 2004), and in laboratory animals as an anti-inflammatory factor for the prevention of glaucoma (Madeira *et al* 2016). Li *et al* (2011) observed that caffeine decreased the chemotaxis and phagocytic activity of neutrophils in pig spermatozoa. Additionally, caffeine has an antibiotic effect against bacteria (Ramanaviciene *et al* 2003, Al-Janabi 2011). Therefore, we suggest that coffee pulp decreases the number of neutrophils in lambs either through the action of caffeine on the adenosine receptors, thereby preventing the inflammatory response, or through its antibacterial activity, which indirectly decreases the amount of neutrophils in the blood.

The addition of up to 14% coffee pulp in the diet increased the number of blood eosinophils above the reference values. Eosinophils are involved in helminth immunity processes (Hogan *et al* 2008, Muniz *et al* 2012, Rosenberg *et al* 2013, Mkrae *et al* 2015). At the end of the experiment, no adult parasites or larval states were found in any treatment. The activation of eosinophils in inflammatory processes and immune response against helminths is regulated by interleukins IL-4, IL-8, IL-13 and TNF-α (Horrigan *et al* 2004, Ciepiela *et al* 2015). Additionally, some receptors are differentially expressed between eosinophils and neutrophils, such as the glucagon-like peptide 1 receptor (GLP-1R) (Mitchell *et al* 2017) and adenosine A3 receptor ($A_{A3}R$) (Ezeamuzie

Table 3. Means (± standard error) of the hematological analysis of lambs fed different levels of inclusion of coffee pulp in the diet.

	Treatment					Reference values
	T0	T1	T2	T3	T4	
Hematocrit	39.47±0.50	40.73±0.50	39.70±0.50	39.75±0.50	39.04±0.55	27-45 %
Leucocyte	9.67±0.44	9.00±0.44	9.04 ±0.44	8.69±0.44	8.78 ±0.48	4-12 x 10^9/L
Neutrophil	4.34±0.19a	4.26±0.19ab	4.01±0.19ab	3.77±0.19b	3.70±0.21b	0.7-6 x10^9/L
Monocyte	0.26±0.06	0.35±0.06	0.27±0.06	0.36±0.06	0.27±0.07	0-0.7 x10^9/L
Lymphocyte	3.52±0.24	3.97±0.24	3.58±0.24	3.22±0.26	3.74±0.25	2-9.0 x10^9/L
Eosinophil	0.95±0.18	0.98±0.18	1.17±0.18	1.14±0.19	1.21±0.20	0-1.0 x10^9/L

T0: control; T1: feed with 7% coffee pulp; T2: feed with 14% coffee pulp; T3: feed with 21% coffee pulp; T4: feed with 28% coffee pulp. Reference values according to Núnez and Bouda 2008.

and Philips 2003), which generates different functional and regulatory consequences). Supplementation with pulp coffee increased eosinophils count, may be pulp coffee activates innate immune response, probably through interleukin, like the findings reported by Oh *et al* (2017). An increase of eosinophils is being associated with an acute stress response or a gastrointestinal inflammation (Weiss and Wardrop 2010, Yantiss 2015). In the present study the lambs did not show clinical signs of any disease

Based on these results, it is concluded that the inclusion of up to 28% coffee pulp in the diet does not affect the productive parameters and does not cause significant changes in the differential leukocyte counts. However, as the pulp concentration increases, neutrophils tend to decrease without affecting the health status of the fattening lambs.

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