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Neem (*Azadirachta indica* A. Juss) leaves as growth promoter in lambs' diets

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ABSTRACT

Objective: To evaluate the dietary inclusion of neem leaves on the productive and biochemical performance of fattening lambs.

Design/methodology: Forty male lambs were assigned according to a completely randomized design. Treatments consisted of dietary inclusion of neem at 0.0, 2.5, 5.0, and 7.5 g kg^{-1} DM for 35 days.

Results: The inclusion of neem leaf in the diet did not affect the productive performance ($P>0.05$), while protein and energy metabolites were modified ($P\leq0.05$).

Limitations on study/implications: The bioactive compounds present in neem modified the metabolites related to protein and energy metabolism, although these changes did not reflect improvements in the productive performance.

Conclusions: The inclusion of dietary neem (2.5-7.5 g kg^{-1}) has no effect on the productive performance, although it does modify some energy and protein metabolites.

Keywords: bioactive compounds, neem, sheep, protein.

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INTRODUCTION

The neem plant (*Azadirachta indica*) is known for its medicinal properties in humans, as antibacterial, antifungal, antiviral, anthelmintic, and hepatoprotector [1]. Meanwhile, in ruminants it is used primarily as fodder and deworming agent [1,2]. Neem contains bioactive compounds such as azadirachtin, nimbidin, nimbidol, salanin and triterpenoid, and depending on the amount ingested, they can be used as growth promoters or become toxic in the diet of ruminants [4].

The assessments that have been made of neem referring to the diet of sheep are based primarily on the use of the seed, the oil and the extract. The incorporation of 3-20% of neem seed paste in lambs' diets did not affect growth, immunity, nutrient digestibility, and meat quality [5,6]. However, the inclusion of 2.5-5% of neem paste in lambs' diets increased the production of microbial protein and improved the efficiency in the use of energy [7], which result in improvements in the consumption of food and weight gain [8]. On the other hand, neem seed oil in ewes' diets modified ruminal fermentation, reduced CH_4 production, and increased digestibility of feed and nitrogen metabolism [9]. The addition of the neem leaf extract (50 ppm) in lambs' diets did not have effects on the characteristics of the canal and the lipid composition of meat [10], although it showed high mortality and inhibition of the incubation of *H. contortus* [3].

However, the incorporation of neem leaves in the diet of lambs has not been widely assessed. Neem leaves contain considerable amounts of carotene, proteins (17-20%), and minerals [1,4], in addition to previously mentioned bioactive compounds. Neem leaves in lambs can be used as sole diet [11], complement or replacement of conventional fodders [4,12]. In addition, neem leaves may be used as antibiotic, anthelmintic, and growth promoter when it is added to sheep's diets [2,3]. Supplementation with neem leaf flour (0.5-1%) in sheep's diets increased weight gain [13]. Meanwhile, the consumption of 3 or 6 g of neem leaf in grazing lambs showed improvements in weight gain [14].

The benefits of the inclusion of neem in ruminants' diets thanks to its content in bioactive compounds represent an alternative to improve the productive performance in fattening lambs. Therefore, the objective of this study was to evaluate the dietary inclusion of neem leaves on the productive and biochemical performance of fattening lambs.

MATERIALS AND METHODS

The experimental procedures were carried out following the ethical norms and animal welfare of Colegio de Postgraduados for the use of animals in experimentation. The experiment was conducted in Colegio de Postgraduados, Campus Montecillo, Estado de México (located at 98° 48' 27" W and 19° 48' 23" N), with mean annual temperature of 15.9 °C and 2241 masl.

Forty male lambs (Hampshire×Suffolk) with initial live weight of 33.6+1.9 kg were randomly assigned in a completely randomized design. The treatments consisted in different dietary concentrations of neem leaves (*Azadirachta indica*) of 0.0, 2.5, 5.0 and 7.5 g kg^{-1} DM in its incorporation to a basal diet (metabolizable energy 2.8 Mcal kg^{-1}, raw protein 147.0 g kg^{-1}, non-degradable protein in rumen 65.9 g kg^{-1}, detergent acid fiber 190.8 g kg^{-1}, calcium 7.0 g kg^{-1} and phosphorus 3.4 g kg^{-1}), formulated in agreement with the recommendations by the NRC [15]. The composition of ingredients (g kg^{-1} MS) of the basal diet was the following: sorghum (322.2), corn (210.0), soy paste (116.8), alfalfa hay (156.2), oat straw (103.8), stubble (40.0), sugarcane molasses (40.0), sodium chloride (1.0), and pre-mixture of vitamins and minerals (10.0). The lambs were housed in individual cages equipped with feeding trough and nipple water dispenser. Before the experiment, the lambs were dewormed (Closantil® 5%) and given vitamins (Vigantol® ADE). The feed was offered at 08:00 and 15:00 h attempting to ensure a rejection of 5-15%. Water and food were provided *ad libitum*. The animals had a period of 7 days of adaptation to the experimental diets. The experimental phase lasted 35 days.

The variables assessed were food consumption (CAL), daily weight gain (DWG), food conversion (FC), and final live weight (fLW). The thickness of the dorsal fat and the *Longissimus dorsi* muscle area were measured using an ultrasound (Sonovet 600 Medison, Inc., Cypress, CA, USA) with a transductor of 7.5 Mhz between ribs 12 and 13 on day 35 of the experiment. The yield of the warm canal was evaluated in five animals per treatment after the sacrifice in a commercial facility.

The last day of the experiment, blood samples were collected (5 mL; pre-prandial 08:00) from the jugular vein by puncture, using vacutainer tubes without anticoagulant (BD Vacutainer) and placed immediately in refrigeration (4 °C). The samples were centrifuged (Sigma 2-16 k, Germany) at 3500 g × 20 min to obtain blood serum, which was stored in Eppendorf tubes and kept in a freezer (Sanyo MDF-436, USA) at −20 °C until its analysis. In each sample, the concentrations determined were: total cholesterol (TC, oxidase-peroxidase enzymatic method); triglycerides (enzymatic method); glucose (enzymatic method); total protein (Biuret method); albumin (green bromocresol method); using specific kits from the Spinreact commercial house (Barcelona, Spain) in a visible UV light spectrophotometer (Cary 1- E Varian, USA). Globulin concentration was calculated by difference between total protein and albumin.

Statistical analysis. The experimental design was completely randomized, four treatments and ten repetitions, considering each lamb as an experimental unit. The Shapiro-Wilk and Levene tests were used to verify the normal distribution and homogeneity of the variance of each variable. The data were analyzed with the GLM procedure, and linear orthogonal and quadratic polynomials ($P \leq 0.05$) were used to determine the effect of the neem intake (Statistical Analysis System 2010. Inc. Cary, NC, USA). The initial weight was used as co-variable for weight gain and final weight ($P \leq 0.05$).

RESULTS AND DISCUSSION

Supplementation with neem in lambs did not cause any change in the final weight, daily weight gain, food consumption or food conversion ($P > 0.05$, Table 1). Regarding the thickness of fat, the area of the ribeye chop and the weight of the canal were also not modified ($P > 0.05$, Table 1) from the effect of the addition of neem to the diet. When it comes to blood metabolites (Table 2), this study shows that the concentrations of glucose, urea, total protein, albumins, and the albumin-globulin rate increased linearly ($P \leq 0.05$), while the concentrations of cholesterol and triglycerides decreased in response to the increase of neem in the diet ($P \leq 0.05$).

Although the incorporation of neem leaf flour (3-6 g kg^{-1} MS) in the diet of fattening sheep has shown a positive effect on the daily weight gain [13,14], in this study, no benefit was found in the final weight and the weight gain when evaluating levels of inclusion of 2.5, 5.0 and 7.5 g kg^{-1} DM. There was a similar performance in goats' diets with the incorporation of neem leaves of up to 12%, since there was no decrease in weight gain [16]. In lambs' diets, the inclusion of 2.5-5% of neem paste modified microbial growth, increasing microbial protein and the efficiency in energy use [7], which indicates that potentially the bioactive compounds of neem could improve the use of nutrients, animal health, weight gain, and stimulate feed consumption [8].

In this experiment, a decrease in the consumption of feed was not observed. The incorporation of neem leaves in goats' diets of up to 12% did not show negative effects on the consumption of feed [16]. Also, in goats' diets with a sole diet of neem leaves, a high voluntary consumption of 3.1% of body weight was seen [11]. The principal limitation that has been reported to use neem in sheep's diets lies in its bitter smell and flavor [14]; in the study conducted, this problem could have been reduced because molasses were added to the diet, which could act as flavoring. Now, the period of 7 days of adaptation could help the animal to accept the diets with high neem content, since if there is dietary insufficiency and an adaptation period, it is possible for ruminants to adapt to the flavor of neem [4].

Regarding the assumption that the higher levels of neem used in the evaluation (2.5, 5.0 and 7.5 g kg^{-1} DM) could present an anti-nutritional effect due to the high consumption of its bioactive components (azadirachtin, azadirone, nimbin, nimbidol, solanine and triterpenoid) [6], it is dismissed, since none of the variables evaluated presented a negative effect. With this, it is possible to speculate that the concentration of bioactive compounds of neem present in the diets assessed do not cause any suppressor effect of the productive performance, with which the additional effects of the consumption of such a plant could be assessed [4, 7, 9].

The changes in blood concentrations of total proteins, globulin albumins, and the albumin:globulin rate could be because the neem leaves have noticeable amounts of proteins (17-20%) [1,4], which could alter the concentrations of the biochemical compounds related to the protein metabolism [4]. In a study with fattening lambs where 30% of the *Brassica campestris* straw was replaced with neem leaves, both the digestibility of the protein, the intake of dry matter and protein, and the production of total volatile fatty acids increased [12]. The inclusion of 5% of neem leaves in sheep's diets increased the serum concentration of protein and urea [2]. At the same time, the biochemical compounds present in neem could modify the protein metabolites, since the inclusion of 2.5-5% of neem paste in lambs' diets modified the microbial growth for a higher production of microbial protein [7]. The influence of the bioactive compounds in the changes of protein metabolites could be confirmed with the study where neem seed oil was supplied (20 ml kg^{-1} DM), which does not contain protein altering the nitrogen metabolism in sheep [9]. However, there is evidence that ewes fed with multi-nutritional blocks with 30% of neem leaves did not alter the serum concentrations of protein, globulin, albumins and urea [12].

The increase of glucose concentration in lambs fed with neem (2.5-7 g kh^{-1} DM) could be because ruminants fed with neem leaf [12], oil [9], and paste [7] increase the amount of volatile fatty acids [7,9], primarily the propionic acid that intervenes in gluconeogenesis. When it comes to the reduction of the levels of cholesterol and triglycerides as the neem concentration increased, the use of neem leaves in poultry production is considered as a hypocholesterolemic dietary additive (which inhibits the synthesis blocking the conversion of desmosterol to cholesterol), and hypotriglyceridemic, reducing the content in meat, fat, and blood serum [17].

CONCLUSIONS

The inclusion of neem in the diet (2.5-7.5 g kg^{-1} DM) does not have effects on the productive performance of lambs; however, it does increase some metabolites related to the use of energy and protein, and reduces the serum levels of cholesterol and triglycerides.

Table 1. Dietary addition of neem on the productive performance of lambs.

Item	Neem (g kg^{-1} de MS)				EE	P	
	0.0	2.5	5.0	7.5		Lineal	Cuadrático
Peso vivo inicial, kg	32.90	33.80	33.42	33.10	0.66		-
Peso vivo final, kg	43.77	45.43	44.88	44.57	0.73	0.72	0.11
GDP kg	0.29	0.35	0.33	0.32	0.02	0.61	0.10
Consumo de MS, kg	1.60	1.67	1.63	1.62	0.03	0.81	0.27
CA	5.56	4.94	4.99	5.21	0.30	0.58	0.08
Grasa dorsal mm	3.29	3.26	3.37	3.22	0.11	0.51	0.47
Área de la chuleta, mm^2	1104	1090	1055	1095	35	0.94	0.20
Peso de la canal caliente, %	46.30	45.56	46.34	44.78	0.89	0.35	0.65

DWG: daily weight gain; FC: food conversion; SE: standard error of the mean.

Table 2. Effect of neem on lambs' metabolites.

	Neem (g kg^{-1} de MS)				EE	P	
	0.0	2.5	5.0	7.5		Lineal	Cuadrático
Colesterol (mg dL^{-1})	70.20	69.60	63.80	61.30	1.97	0.01	0.63
Triglicéridos (mg dL^{-1})	41.28	33.56	32.81	34.95	1.87	0.02	0.01
Glucosa (mg dL^{-1})	66.40	74.50	67.40	81.40	4.19	0.05	0.48
Urea (mg dL^{-1})	27.90	30.50	34.40	34.00	1.76	0.01	0.40
Proteína total (g dL^{-1})	7.81	8.74	9.68	10.02	0.18	0.01	0.11
Albuminas (g dL^{-1})	3.62	4.66	5.56	5.82	0.13	0.01	0.39
Globulinas (g dL^{-1})	4.19	4.08	4.12	4.20	0.11	0.89	0.41
Albuminas/globulinas	0.86	1.15	1.36	1.40	0.05	0.01	0.12

SE: standard error of the mean.

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