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Evaluation of herbal choline in productive performance and blood metabolites of ewes

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ABSTRACT

Objective: To evaluate the effect of different levels of protected herbal choline (CHP) on productive performance, carcass characteristics, and serum metabolites in ewes.

Design/Methodology/Approach: Fifty-two Hampshire × Suffolk ewes (29.95±3.90 kg of initial body weight, IBW) were randomly assigned to one within four treatments: 0, 0.3, 0.6 and 0.9% choline kg^{-1} dry matter (DM). The experimental design was complete randomized, in order to detect linear or quadratic trends.

Results: No treatment effects were detected in the production variables, dorsal fat thickness and *Longissimus dorsi* muscle area, and AML with the addition of protected herbal choline in the diet ($p>0.05$). CHP linearly increased the concentration of cholesterol, glucose, albumins, globulins, total proteins ($p\leq0.05$) and phosphatidylcholine ($p\leq0.10$). The triglyceride concentration had a quadratic response ($p\leq0.05$) to the addition of CHP.

Study limitations/Implications: The level of choline supplementation in sheep depends on whether the source is herbal or synthetic.

Findings/Conclusions: The addition of CHP in ewe diets raised the concentration of phosphatidylcholine, modified the concentration of protein and lipid metabolites. However, no improvements in production were found.

Keywords: sheep, energy metabolites, herbal choline.

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INTRODUCTION

Choline is considered a metabolically essential B-complex vitamin in sheep (NRC, 2007). Choline metabolites in the body are important for the synthesis of proteins, phospholipids, acetylcholine, bone growth; also, as an essential factor in fat metabolism in the liver and methylation processes (NRC, 2007).

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Dietary choline is extensively degraded in the rumen and a limited amount passes to the intestine (Baldi and Pinotti, 2006). Sources of protected choline are available on the market; however, in the feeding of lambs are not included regularly. The addition of protected choline in the diet of ruminants may reduce choline deficiencies and improve productive performance, health, and reproduction (Pinotti *et al.*, 2009; Jayaprakash *et al.*, 2016; Gutierrez *et al.*, 2019; Mendoza *et al.*, 2020).

Choline requirement in sheep has not been clearly established, but dietary supplementation may enhance productive performance (NRC, 2007). There is evidence that the addition of protected choline improves productive performance in lambs in completion, modifies hormones related to fat and protein metabolism, and stimulates the synthesis of glucose and cholesterol (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018).

Choline chloride is the synthetic source of choline commonly used in animal diets. However, under poor storage conditions, high hygroscopicity can accelerate the oxidation of food vitamins and premixes (Tavcar-Kalcher and Vengust, 2007). On the other hand, the use of levels higher than 3 g d^{-1} $animal^{-1}$ of protected choline chloride (CCP) would have adverse or no effects on the productive behavior of fat sheep (Bryant *et al.*, 1999; Li *et al.*, 2015), due to its low bioavailability (40-80%) and toxicity of secondary metabolic products (Sharma and Erdman, 1989; Jayaprakash *et al.*, 2016).

In addition, organic animal production is restricting the use of synthetic compounds such as choline chloride. This is the reason why researchers seek for alternative-natural dietary supplements in diets for ruminants.

Previous studies with fattening lambs (Godínez-Cruz *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2019) have demonstrated the possibility of replacing CCP with protected herbal choline (CHP), because CHP contains phospholipids (mainly phosphatidylcholine) instead of choline chloride. The metabolic pathway of phosphatidylcholine in the body is different from free choline, as phosphatidylcholine requires less energy expenditure and does not require various metabolic processes to be available to cells. Free choline requires transporters to enter cells (some require ATP), then requires an ATP molecule for phosphocholine formation, followed by the conversion of phosphocholine to cytidine-di-phosphocholine, which determines the biosynthetic flow of choline to phosphatidylcholine (Fagone and Jackowski, 2013).

Supplementation with CHP in diets for ewes in completion could be a strategy to make metabolic and methylation processes more efficient, being an alternative source to the use of protected choline chloride. Therefore, the objective of this study was to evaluate the effect of different levels of CHP on productive performance, carcass characteristics and serum metabolites in ewes.

MATERIALS AND METHODS

Location of the study

The experiment was implemented following the standards of ethics, biosecurity and animal welfare of the Colegio de Postgraduados (CP), under the Official Mexican Standard NOM-062-ZOO-1999 at the CP Experimental Farm, located in Montecillo, State of

Mexico (19° 27' 38" N, 98° 54' 11" O, 2250 m). The climate is sub-humid temperate, with average annual temperature and rainfall of 15.8 °C and 663.7 mm, respectively.

Animals and treatments

The experiment used fifty-two Hampshire × Suffolk ewes (30.36±3.75 kg BW and four-month-old) housed in individual cages equipped with a feeder and drinker. The study factor was the dietary supplementation of Biocholine Powder® (Nuproxa Mexico, Querétaro, Mexico), a supplement containing choline of herbal origin with 16 g kg^{-1} of choline conjugates and is a polyherbal mixture based on *Achyrantes aspera*, *Trachyspermum ammi*, *Azadirachta indica*, *Citrullus colocynthis* and *Andrographis paniculata*. In each treatment, 13 ewes were randomly assigned, in a complete randomized design with four treatments. The treatments consisted of different dietary concentrations of Biocholine Powder® of 0, 3, 6 and 9 g kg^{-1} base MS in its incorporation of a basal diet (metabolizable energy 2.9 Mcal kg^{-1}, crude protein 189.0 g kg^{-1}, non-degradable protein in rumen 75.2, detergent acid fiber 150.5 g kg^{-1}, calcium 5.0 g kg^{-1} and phosphorus 4.6 g kg^{-1}) formulated in accordance with the recommendations of the NRC (2007). The composition of ingredients (g kg^{-1} MS) of the basal diet was as follows: corn (567.8), soybean paste (227.3), alfalfa hay (100.5), oat straw (50.0), cane molasses (40.0), calcium carbonate (2.4), sodium chloride (2.0) and a vitamin plus mineral premix (10.0).

The ewes were adapted to the basal diet for a period of 8 d the experimental phase lasted 60 days. The feed was offered at 08:00 and 16:00 h and the water *ad libitum*. The production variables were dry matter intake (DMI, kg d^{-1}), daily weight gain (DWG, g d^{-1}), feed conversion (FC) and final body weight (FBW). The average DWG was calculated with the initial body weight (IBW) and final body weight (FBW) of the experiment during the fasting period. The dorsal fat thickness and *Longissimus dorsi* muscle area were measured using a Sonovet 600 real-time ultrasound (Medison, Inc., Cypress, California, USA) with a 7.5 Mhz transducer between the 12^{th} and 13^{th} rib, on days 1 and 60 of the experiment.

At the end of the experiment (08:00 preprandial) 5 mL of blood were collected, by puncture of the jugular vein, in a tube without anticoagulant (BD Vacutainer®) for serum separation and placed in refrigeration (4 °C). The samples were centrifuged (Sigma 2-16 k, Germany) at 2500 x *g* for 20 min to obtain blood serum and stored in Eppendorf tubes at −20 °C until further analysis. In each sample, the concentration of total cholesterol (enzymatic oxidase-peroxidase method) was determined; triglycerides (enzyme method); high-density lipoproteins (HDL, enzyme method); phosphatidylcholine (enzymatic method); glucose (enzymatic method); total protein (Biuret method) and albumin (bromocresol-green method) using specific kits from the Spinreact trademark (Barcelona, Spain). By difference between total proteins and albumin, the blood concentration of globulins was obtained.

Statistical analysis

A complete randomized design was used, with four treatments and 13 replicates considering each ewe as an experimental unit. The Shapiro-Wilk and Levene tests were used to verify the normal distribution and homogeneity of the variance of the variables.

Data were analyzed using PROC GLM (SAS, 2010) and for the effect of choline intake orthogonal polynomials were used to detect linear or quadratic responses. Initial body weight was used as a covariate.

RESULTS AND DISCUSSION

Productive performance

The inclusion of protected herbal choline in the diet had no effect on the productive variables, dorsal fat thickness and *Longissimus dorsi* muscle area ($p>0.05$, Table 1) of the ewes.

Generally speaking, the dietary inclusion of choline in ruminants has beneficial effects. The addition of CCP in the diet of dairy cows improved productive performance during the postpartum period (Baldi and Pinotti, 2006); increased production and improved milk composition, and reproductive parameters (Jayaprakash *et al.*, 2016). In cattle and goats, feed conversion and weight gain were improved (Pinotti *et al.*, 2009; Habeeb *et al.*, 2017). However, when very high levels of choline chloride were used, no improvements in productive performance were observed (Pawar *et al.*, 2015; Budiarsana *et al.*, 2016).

In this study, the intake of 4.65-14.76 g d^{-1} of CHP had a null efect on the productive variables and the carcass characteristics. Which is consistent with the results observed in other studies (Godínez-Cruz *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018), where the supplementation of 4 g d^{-1} of CHP in the diet of finishing lambs did not modify the productive performance or the carcass characteristics. Is in contrast to some studies where the inclusion of CHP in ruminants may improve, the overall health of dairy cattle (Gutiérrez *et al.*, 2019; Mendoza *et al.*, 2020), and the productive performance of fattening lambs (3, 6 and 9 g kg^{-1} of DM; Martínez-Aispuro *et al.*, 2019).

In contrast, Li *et al.* (2015) found that production parameters and carcass characteristics responded quadratically to the inclusion of SPCs in lamb diets, observing the best response with 2.6 g d^{-1}. However, adverse effects were found with 7.8 g d^{-1} of CCP on daily weight gain. Bryant *et al.* (1999) observed increase in daily weight gain by suplement 2 g d^{-1} of CCP in the lamb diet but suplement 4 and 10 g d^{-1} of CCP, no additional benefit

Table 1. Productive variables of ewes fed with a diet supplemented with herbal choline.

Item	Herbal choline (g kg^{-1} DM)†				SEM	P-value	
	0	3	6	9		Linear	Cuadratic
Initial BW, kg	29.69	30.01	30.09	30.02	1.11	-	-
Final BW, kg	46.37	46.06	46.82	45.94	0.69	0.86	0.81
DWG, g d^{-1}	0.293	0.289	0.300	0.287	0.012	0.87	0.82
DMI, kg d^{-1}	1.54	1.55	1.55	1.64	0.06	0.57	0.52
Feed conversion	5.30	5.39	5.18	5.50	0.06	0.57	0.58
Backfat, mm	4.52	4.64	4.60	4.46	0.15	0.74	0.63
Chop area, mm^2	1107	1125	1108	1132	21	0.53	0.53

† Biocholine Powder® (Nuproxa Mexico); IBW: Initial body weight; FBW: final body weight; DWG: daily weight gain; DMI: dry matter intake; FC, feed conversion; SEM: standard error of the mean.

was observed. Thus, it is inferred that the use of choline chloride levels above 3 g d^{-1} has adverse or no effects on the productive performance of fattening lambs (Bryant *et al.*, 1999; Li *et al.*, 2015). However, Kawas *et al.* (2020) when supplementing 0, 0.1, 0.2, and 0.3% CCP (concentration of 25%) in fattening lambs, did not observe improvements in productive performance, although obtaining an increase in dorsal fat.

The difference in dietary supplementation tolerance between CCP and CHP is related to the presence of secondary compounds because choline chloride has an *in vitro* or *in situ* degradation between 40 to 80%, and the rest can be converted to trimethylamine (toxic compound) by intestinal bacteria (Sharma and Erdman, 1989; Jayaprakash *et al.*, 2016). Whereas CHP does not show this problem, since the phosphatidylcholine present in herbal choline is a source of esterified choline conjugated to a phosphate molecule, which is more active and bioavailable than choline chloride (Fagone and Jackowski, 2013).

In dorsal fat thickness and *Longissimus dorsi* muscle area, the addition of herbal choline had no effect, which coincides with other experiments by including levels of 2-10 g d^{-1} of protected choline (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015).

Another possible explanation for the lack of response to CHP supplementation in this research is that only females were used, whereas males and females were used in previous studies (Bryant *et al.*, 1999; Godínez-Cruz *et al.*, 2015; Li *et al.*, 2015; Rodríguez-Guerrero *et al.*, 2018). Studies in people reported a marked differentiation in methylation processes between males and females (McCarthy *et al.*, 2014); which would lead to think that the choline requirement is different between sexes.

Serum metabolites

The addition of CHP linearly increased the concentration of cholesterol, glucose, total proteins, albumins, globulins ($p<0.01$) and phosphatidylcholine ($p<0.08$) (Table 2). The concentration of triglycerides presented a quadratic response ($p<0.05$) to the addition of CHP. The lowest concentration of this metabolite was recorded with the use of 4.5 and 9.3 g d^{-1} of CHP. The addition of CHP did not affect the HDL concentration and the albumin/globulin ratio in blood serum ($p>0.10$).

In this study, CHP supplementation showed a linear increase in phosphatidylcholine in plasma, confirming that CHP supplementation was effective in incorporating choline into the lamb metabolism (Martínez-Aispuro *et al.*, 2019). Similar to what was reported by Habeeb *et al.* (2017) who found that choline supplementation in goats increased plasma concentrations of the metabolite.

Choline supplementation has been documented to show a linear trend to increase blood glucose in cows and goats (Zhou *et al.*, 2016; Habeeb *et al.*, 2017). In lambs, it is observed that the inclusion of herbal choline in the diet increases the concentration of glucose in blood serum (Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2019). Choline could alter intracellular signaling of energy metabolism, as it occurs in cases of insulin resistance, where choline supplementation reduces glucose utilization for fatty acid and triglyceride synthesis and increased muscle glycogen (Taylor *et al.*, 2017).

Table 2. Blood metabolites in ewes fed a diet supplemented with herbal choline.

Item	Herbal choline (g kg^{-1} DM)†				SEM	P-value	
	0	3	6	9		Linear	Cuadratic
Cholesterol, mg dL^{-1}	96.10	103.55	118.76	114.83	3.59	0.001	0.01
PCho, mg dL^{-1}	106.14	108.40	116.11	115.10	8.24	0.08	0.23
Triglycerids, mg dL^{-1}	32.27	26.55	28.82	30.95	1.87	0.84	0.04
HDL, mg dL^{-1}	55.07	56.13	55.24	55.68	1.67	0.91	0.85
Glucose, mg dL^{-1}	75.09	80.82	83.61	86.08	2.85	0.008	0.57
Total protein, g dL^{-1}	5.75	5.81	7.58	7.91	0.39	0.001	0.73
Albumins, g dL^{-1}	2.78	2.81	3.17	3.50	0.12	0.001	0.27
Globulins, g dL^{-1}	2.98	3.00	4.40	4.42	0.36	0.001	0.98
Albumins/ Globulins	0.94	0.96	0.80	0.87	0.06	0.20	0.68

† Biocholine Powder® (Nuproxa Mexico); PCho: Phosphatidylcholine; HDL: high-density lipoproteins; SEM: standard error of the mean.

Similar to the results found in this study, the addition of 4 g d^{-1} of herbal choline in lamb diets increased serum cholesterol (Rodríguez-Guerrero *et al.*, 2018). In dairy cows, choline supplementation significantly increased cholesterol concentration (Soltan *et al.*, 2012). Nevertheless, the consumption of CCP (2-10 g d^{-1}) in lamb diets did not change serum cholesterol concentration (Bryant *et al.*, 1999; Li *et al.*, 2015). Choline is necessary for the transport and metabolism of lipid cholesterol (Zeisel and Costa, 2009), which could explain the changes in blood cholesterol concentrations.

A deficiency of choline in muscle cells leads to the accumulation of triglycerides in cattle (Bryant *et al.*, 1999). Similar to what was found in this study, choline supplementation in goats reduced triglyceride concentration (Habeeb *et al.*, 2017; Rodríguez-Guerrero *et al.*, 2018; Kawas *et al.*, 2020). However, in other studies the consumption of 2-10 g d^{-1} of choline did not modify the serum concentration of triglycerides in lambs (Bryant *et al.*, 1999; Li *et al.*, 2015).

Consistent with the results obtained in this study, choline supplementation in goats and fattening sheep increased globulin concentrations (Habeeb *et al.*, 2017; Martínez-Aispuro *et al.*, 2019). Whereas, in lambs supplemented with CHP (4 g d^{-1}), it did not modify serum albumin or total protein concentration (Rodríguez-Guerrero *et al.*, 2018).

Although in this research the concentrations of high-density lipoproteins were not modified; there is conflicting evidence that choline supplementation (2.6 g d^{-1} of CCP) to fattening lambs reduces (Li *et al.*, 2015) or does not affect (with CHP, Martínez-Aispuro *et al.*, 2019) the concentration of high-density lipoproteins.

CONCLUSIONS

Herbal choline is effective in raising the concentration of phosphatidylcholine in the body of ewes during fattening. It also modified the concentration of protein, lipid and energy metabolites. However, the consumption of 4.5-14.0 g d^{-1} of herbal choline does not favor the productive performance.

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