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Changes in Productive Performance, Blood Metabolites and Hematological Parameters of Growing Lambs Supplemented with Two Sources of Choline

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ABSTRACT

Background: Choline is a nutrient with numerous metabolic functions, but its requirements for ruminants are unknown. The supplementation with bypass choline could enhance productivity.

Methods: Twenty-four male lambs (Rambouillet 23.5 kg±3.17 kg initial BW) were fed a basal diet with treatments which consisted of a control and oral doses of ruminally-protected choline (4 g/d RPC) and plant-based choline (4 g/d Biocholine) in a completely randomized design with initial weight as a covariate. The experiment was conducted for 42 days during which live weight, dry matter intake, carcass characteristics, blood metabolites and basic hemograms were measured.

Result: The daily gain in lambs was similar between treatments. Intake was higher in lambs given Biocholine (1.32 kg/d). The L* (represents the light to dark color) value and mineral content in the meat were improved with both sources of choline. Blood triglycerides increased by RPC compared with the other treatments and cholesterol was reduced by Biocholine. Alanine transaminase (ALT) and aspartate aminotransferase (AST) activity decreased by effect of choline. Hematological parameters were affected by choline supplementation regardless of the source; erythrocyte, monocytes and lymphocytes count decreased with both sources of choline in growing lambs.

Key words: Carcass, Choline, Growth meat, Health, Lamb.

INTRODUCTION

Choline has important functions in energy metabolism as a lipotropic factor and in DNA synthesis as a donor of methyl groups (Zhu *et al*., 2014; Roque-Jimenez *et al*., 2020). Biswas and Giri (2015) described antioxidant capacity and protective properties of choline against some toxic agents, while other studies (Tőkés *et al*., 2015) have indicated that dietary choline phospholipids such as phosphatidylcholine may also have anti-inflammatory effects or to reduce fatty liver condition (Acharya *et al*., 2020).

Although the choline requirements of sheep are unknown (NRC, 2007), earlier studies have indicated that supplementation with ruminally-protected choline (RPC) improves lamb growth (Bryant *et al*., 1999). Recent studies have confirmed that weight gain can be improved in lambs (Li *et al*., 2015) with an estimated dose of 37 mg/kg BW0.75 of bypass choline. However, the use of commercial RPC requires knowing its choline content and the bypass fraction to establish the desired dose. *In vitro* ruminal incubations indicate that ruminal protection varies from 63 to 98% (Kung *et al*., 2003). Previous studies with lambs demonstrated that an herbal product could substitute RPC as a choline source (Godinez-Cruz *et al*., 2015). Information on herbal sources of choline in ruminants is scarce compared with choline chloride rumen-protected forms (Li *et al*., 2015) for which bypass has been estimated (Kung *et al*., 2003). Most of the choline in herbal sources is found in the lipid fraction as total conjugates, which prevent rumen degradation (Godinez-Cruz *et al*., 2015, Khose *et al*. 2019). The lipid fraction of choline is mainly in the form of phosphatidylcholine (Zeisel *et al*., 2003). Therefore, the objective of this experiment was to compare choline from an herbal source versus a ruminally-protected source on

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performance, carcass characteristics, meat quality, hematological and biochemical plasma values in finishing lambs.

MATERIALS AND METHODS

All animal management procedures were conducted following the set of regulations and standards that are required by the Mexican government for the use of animals for diverse activities, as well as the guidelines of the Academic Committee, under the State Law on Animal Protection for the State of San Luis Potosi, Mexico (ONSSLP, 2014).

Animals

Twenty-four male lambs (Rambouillet 23.48 kg±3.17 kg initial BW) were randomly assigned to one of three dietary treatments (n = 8 lambs/treatment): a) control group; b) oral dose of ruminally-protected choline (4 g/d RPC, Balchem Reashure); c) oral dose of plant-based Biocholine (4 g/d Nuproxa Mexico; Indian Herbs). Supplementation of choline from the two sources was top dressed on the feed every day. Lambs were fed in individual pens with a basal diet (Table 1; NRC, 2007).

The rations were ground (using a 1.25 cm screen) and mixed in a grinder-mixer (Vigusa, Mexico) and provided *ad libitum* in individual feeders (50 cm bunk space/lamb) at 08:00 and 15:00 h, ensuring 100 g feed refusal daily.

Table 1: Ingredients and chemical composition of the experimental diets.

Item	Ingredient, % DM
Sorghum grain (cracked)	17.0
Sorghum grain (ground)	13.0
Oat grain	17.2
Soybean meal	13.9
Cane molasses	5.0
Alfalfa hay	30.0
Minerals[1]	2.0
Sodium bicarbonate	2.0
Nutrient composition	
Dry matter %	88.0
Crude protein %	16.52
aNDF %	29.1
aADF %	19.2
Ether extract %	3.43
Ca %	0.66
P %	0.33
Metabolizable energy Mcal/kg	2.52

[1] Commercial Vitasal Engorda Ovinos Plus, each kilogram of minerals contained: Ca 27 g, P 3 g, Mg 0.75 g, Na 6.56 g, Cl 10 g, K 0.05 g, S 42. ppm, lasalocid 2,000 ppm, Fe 978 ppm, Zn 3,000 ppm, Se 20 ppm, Co 15 ppm, vitamin A 35,000 IU, vitamin D 150,000 IU and vitamin E 150 IU. aNDF: neutral detergent fiber and aADF: acid detergent fiber.

Feed samples collection

One sample of feed per week was collected, dried in forced-air oven at 60°C until constant weight was reached and stored for analysis. All samples were dried to calculate dry matter (DM) and crude protein (CP) according AOAC (1995) methodology. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were carried out following Van Soest *et al*. (1991) using the filter bag technique with addition of sodium sulfite and heat-stable amylase to determine NDF.

Finishing and carcass data collection

Daily feed intake was calculated as feed offered minus feed refused, measured daily before the morning feeding. The lambs were weighed at the beginning and at the end of the experiment after an adaptation period to estimate average daily gain. Feed conversion was expressed as the ratio of feed intake to average daily gain.

After the 46-d feeding period, all lambs were slaughtered immediately in a commercial abattoir. After slaughter hot carcass was recorded. After chilling for 24 h at 4°C, cold carcass weight was obtained.

Meat analysis

Color was measured 24 h after slaughter in fresh cuts of loin (Longissimus dorsi muscle) samples using a Minolta CM-2006d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan). Luminosity (L*), redness (a*) and yellowness (b*) were recorded (Ripoll *et al*., 2012). Muscle samples (approximately 5 g) were collected and stored in a freezer (-20°C) until analysis. To measure meat tenderness a model 1132 Instron Universal Testing Machine (Instron, Canton, MA) with a Warner-Bratzler attachment (Wheeler *et al*., 1997) was used. Moisture, protein, ether extract and ash contents were analyzed in samples of Longissimus dorsi following AOAC (1995) procedures.

Blood collection and analysis

Blood samples were collected from all lambs after fasting (2 h) by jugular vein puncture using tubes without anticoagulant on days 7, 14, 21, 28 and 35 for hematological assessment were measured with an electronic analyzer (ABXMicros 60, Horiba ABX, France). Serum samples from day 35 were used to analyze glucose (glucose oxidase and peroxidase method), total protein (biuret method), triglycerides (glycerol 3-phosphate oxidase-PAP method) and cholesterol (cholesterol ester hydrolase, cholesterol oxidase and peroxidase) using Sigma diagnostic kits and alanine transaminase activity (GPT (ALT) Spinreact México) and aspartate aminotransferase activity (GOT (AST) Spinreact México).

Statistical analysis

Results were analyzed according to a completely randomized design using initial body weight as a covariate to the performance variables (average daily ganin, dry matter intake, feed conversion hot carcass weight and cold carcass

weight). An ANOVA was performed and means were compared with the Tukey test (Steel *et al*., 1997). Variables measured more than once (hematological values) were analyzed as repeated measures over time (Littell and Henry 1998). Data were analyzed using JMP7 software (Sall *et al*., 2012).

RESULTS AND DISCUSSION

The plant-based choline improved (P<0.05) daily gain, relative to the control group and RPC group (Table 2). In this experiment, intake was highest (P<0.05) in lambs with Biocholine. An experiment by Li *et al*. (2015) indicates that higher doses of bypass choline may have a detrimental effect on gain in lambs; same authors did not detect differences in daily feed intake.

Some authors evaluated RCP where they found that intake of growing steers was not affected (Pinotti *et al*., 2009; Hajilou *et al*., 2014). But, due to the metabolism changes during lactation, a significant effect of choline on dry matter intake could be observed in dairy cows (Sales *et al*. 2010).

Some RCP evaluations showed that intake of growing steers was not affected (Pinotti *et al*., 2009; Hajilou *et al*., 2014) and Sales *et al*. (2010) indicates that few studies have found any significant effect of dietary RCP on dry matter intake of dairy cows.

Cold carcass weight was highest (P<0.05) in the lambs that received Biocholine. Shear force of meat was not affected (P<0.05). Meat brightness increased (P<0.05) with the two choline sources. The mineral content in the meat was increased (P<0.05) with both sources of choline, but the protein level was reduced with Biocholine. Shear force of the meat in our study contrasts with what was reported by Li *et al*. (2015), who found that shear force was reduced by 0.25% in lambs with RPC. However, Li *et al*. (2015), did not observe changes in intramuscular ether extract in lambs fed with different levels of RPC. In the case of color enhancement in lambs fed the herbal product, Jiao *et al*. (2019) found that Biocholine changed meat color of supplemented finishing pigs. Moreover, choline itself has antioxidant effects that have been observed in pork L* values from pigs fed supplemented choline (Li *et al*., 2015), similar that Kumar *et al*. (2018) observed on Barandur rambs.

Factors that affect the mineral content in meat have received little attention. The values observed in our control group (1.96%) are like those reported (1.16%) in finishing diets (Vnučec *et al*., 2016). Methyl donors could have affected protein and lipid partitioning in animal bodies (Schrama *et al*., 2003), accelerated synthesis of myoglobin and increased fat deposit in longissimus muscle. Puchala *et al*. (1995) reported that the amino acid composition in sheep serum was changed by the methyl donor and some changes in amino acid composition in longissimus muscle of pigs were reported by Yu *et al*. (2004).

Blood triglycerides (Table 3) were increased by RPC compared with the effect of Biocholine and the control group. Cholesterol was reduced by Biocholine but was not affected by RPC. Cholesterol concentrations were associated with the lipotropic functions of phosphatidylcholine (Cole *et al*., 2012) because choline increases hepatic lipid transport (Zhu *et al*., 2014). Bindel *et al*. (2000) showed that triglyceride blood concentrations in heifers increased in response to RPC, but only when tallow was included in the diet. In a metabolic experiment conducted by Bindel *et al*. (2005) on steers where choline was infused abomasally (4 g/d), triglyceride levels were reduced by 15% (with or without tallow), whereas cholesterol was reduced by 8.8% only in

Table 2: Effect of choline source on lamb performance and carcass characteristics.

Variable	Control	Biocholine	RPC	SEM[1]	*P*-value
Initial BW (kg)	22.65^{a}	25.97^{b}	25.92^{b}	0.65	0.04
Final BW (kg)[2]	35.12^{b}	37.98^{c}	33.78^{a}	1.21	0.01
ADG (kg)[2]	0.29^{a}	0.32^{b}	0.22^{a}	0.01	0.01
DM Intake (kg/d)	1.26^{ab}	1.32^{b}	1.06^{a}	0.04	0.01
Feed conversion	4.32^{b}	4.15^{a}	4.76^{c}	0.13	0.01
Hot carcass weight (kg)	18.07	19.15	17.21	0.60	0.09
Cold carcass weight (kg)	17.44^{ab}	18.61^{b}	16.53^{a}	0.55	0.04
Color					
*L**	40.90^{a}	42.94^{b}	43.08^{b}	0.50	0.01
a*	21.09	20.76	20.68	0.29	0.57
b*	5.82	6.13	6.04	0.31	0.78
Shear force (kg/cm^{2})	26.95	26.89	32.16	4.94	0.69
Moisture (%)	73.35	75.21	73.35	0.58	0.10
Fat (%)	12.37	11.42	12.55	1.56	0.74
Protein (%)	54.93^{b}	45.52^{a}	53.78^{b}	0.22	0.01
Ash (%)	1.96^{a}	3.99^{b}	3.79^{b}	0.60	0.01

^{a,b,c} Values with different letters in a row are different P<0.05), [1] SEM = Standard error of mean, L* represents the light to dark color, a* represents green to red and b* represents the blue and yellow tones.

Table 3: Effect of choline source on blood metabolites.

Parameters	Control	Biocholine	RPC	SEM[1]	*P*-value
Glucose (mg/dl)	88.77	88.06	83.28	0.78	0.11
Cholesterol (mg/dl)	25.75[b]	19.51[a]	27.07[b]	1.31	0.01
Triglycerides (mg/dl)	123.56[a]	125.01[a]	134.3[b]	1.23	0.01
Plasma protein (mg/dl)	62.00	50.70	64.50	7.75	0.21
AST[2] (IU)	116.2[c]	70.88[a]	89.50[b]	3.91	0.01
ALT[3] (IU)	15.23[ab]	16.47[b]	13.51[a]	0.46	0.02

[a,b,c] Values with different letters in a row are different (p<0.05); [1] SEM = Standard error of mean; [2] AST = aspartate aminotransferase; [3] ALT = alanine transaminase.

Table 4: Effect of choline source on red and white blood counts in lambs during the experiment.

Parameter/day	Control	Biocholine	RPC	SEM[1]	Treatment	Time	Time x Treatment
Erythrocytes × 10^6/ml							
7	3.40	3.40	3.17	0.07	0.17	0.09	0.27
14	3.57[ab]	3.40[a]	3.75[b]	0.07	0.04	0.18	0.13
21	3.70	3.70	3.50	0.07	0.12	0.15	0.16
28	3.65[b]	3.65[b]	3.45[a]	0.04	0.05	0.10	0.37
35	3.75[b]	3.55[a]	3.55[a]	0.05	0.03	0.58	0.29
Leukocytes /ml							
7	10100	7870	1040	844	0.21	0.02	0.25
14	11300	9050	8770	1155	0.59	0.04	0.08
21	12000	8800	9900	1074	0.35	0.03	0.16
28	8320	8320	8400	853	0.20	0.02	0.10
35	9700	9700	9070	839	0.36	0.06	0.18
Monocytes/ml							
7	77	74	139	23.1	0.87	0.05	0.10
14	115	155	82	25.8	0.50	0.04	0.63
21	342[b]	128[a]	153[ab]	53.9	0.05	0.07	0.07
28	191	111	166	58.7	0.92	0.03	0.22
35	223	203	187	67.5	0.59	0.08	0.46
Lymphocytes/ml							
7	5030[ab]	4290[a]	6220[b]	453	0.04	0.02	0.09
14	7190[b]	5170[ab]	4140[a]	788	0.05	0.05	0.12
21	6620	4820	5530	604	0.82	0.02	0.14
28	4960	4230	4490	500	0.79	0.09	0.41
35	5460	4650	5430	286	0.17	0.08	0.34

[a,b,c] Values with different letters in a row are different (p<0.05); [1] SEM: standard error of the mean.

steers fed a diet containing tallow. Hajilou *et al*. (2014) used a low-fat diet and reported a reduction in triglycerides with RPC in young Holstein bulls. In growing lambs, Bryant *et al*. (1999) reported increased triglyceride concentrations and a tendency toward decreased serum cholesterol concentrations when RPC was included in a diet that included yellow grease.

The levels of the liver enzyme AST differed among all treatments (P<0.05), as it was reduced by both sources of choline relative to the control group. The ALT was higher (P<0.05) for Biocholine. Choline has been demonstrated to have liver protective effects (Zhu *et al*., 2014) and it has been shown to improve the integrity and signaling functions of cell membranes (Fagone and Jackowski, 2009). Supplementation with choline has been shown to reduce some hepatic enzyme levels, indicating improved hepatic function (Rahamani *et al*., 2014), whereas choline-deficient diets increase liver enzyme activity (Getty and Dilger, 2015) and cause liver damage in several species (Guo *et al*., 2005). Erythrocyte counts (Table 4) showed differences on days 14, 28 and 35 of the experiment. Comparing choline from the two sources, on day 14, erythrocyte counts were lower in the Biocholine group, but in the RPC group they were lower on day 28 (P<0.05) and similar on day 35. For lymphocyte counts, on day 14 the lowest (P<0.05) values were found in the RPC group. Regarding erythrocyte counts, non-clinical anemia is a common anomaly observed in ruminant blood profiles, with a hematocrit below 24%,

erythrocyte count below 5x106 cells/mL or Hgb concentrations below 8 g/dL (Cole *et al*., 1997). For leukocytes, no differences (P<0.05) in their total count volume were found between treatments; all values were in the normal range from 4,000 to 12,000 (106 x µL). Monocytes alone showed an increase (P<0.05) in the control group on day 21. There is no biological explanation for these values, although the analysis shows differences. The values for lymphocytes (2,000 to 9,000/mL) were normal in all treatments since the lowest value was 4,150 and the highest was 7,190/mL.

No lymphocytosis or lymphopenia was observed (Jones *et al*., 2007). Due to the function of choline as a component of the platelet activating factor (Prescott *et al*., 2000; McIntyre *et al*., 2009), in experimental endotoxemia a positive response in terms of platelet count and a return to the initial WBC were demonstrated following intravenous administration of choline chloride or cytidine-5'-diphosphate choline (Prescot *et al*., 2000). However, in an experiment investigating choline deficient and sufficient diets, no changes were observed in erythrocytes in piglets (Schrama *et al*., 2003). The response in these cells may depend on the physical condition of the animal, as studies in dairy cattle indicate that mastitis and morbidity can be reduced with RPC (McIntyre *et al*., 2009).

CONCLUSION

Daily gain and cholesterol concentrations had better results with choline supplementation. In turn, the lowest levels of triglycerides were observed in the control group. Both sources of choline modified meat color and increased its mineral content. ALT and AST activity decreased by effect of both source of choline. Hematological parameters were affected by choline supplementation there was a reduction in their counts with choline from both sources in growing lambs.

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