

## Evaluation of polyherbal methionine and choline in feedlot rations for lambs

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### Abstract

Requirements of lambs for choline and methionine have not been clearly established, but because of their metabolic relationship, the availability of these two nutrients may affect protein synthesis and energy balance. Therefore, a trial was carried out to evaluate the effect on productive performance and blood metabolites of including methionine and choline from polyherbal mixtures in finishing lamb diets. Forty Hampshire x Suffolk lambs weighing  $26.9 \pm 2.8$  kg were used for a 45-day experiment. The treatments were arranged as a 2x2 factorial, in which the factors were dietary supplementation with herbal methionine and choline at two levels (0% and 0.4% of dry matter (DM)). The treatments had no effects on the lambs' productive variables (daily feed intake, average daily gain, and feed to gain ratio), carcass characteristics and lipid metabolites ( $P > 0.05$ ). The results indicated that the inclusion of polyherbal mixtures containing methionine, choline and their combination at 0.4% of DM in finishing lamb diets showed no benefits in productive response or in blood metabolites related to lipid metabolism.

**Keywords:** amino acid, lipid metabolites, productive performance, ruminally protected, vitamin

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### Introduction

Methionine is one of two limiting amino acids for protein synthesis in growing lambs, especially when a diet is high in ruminally degradable protein (Nolte *et al.*, 2004; El-Tahawy *et al.*, 2015). Under this condition, Osorio *et al.* (2013) and Lapierre *et al.* (2009) suggested that microbial protein that is synthesized in the rumen must be complemented with ruminally undegradable protein or protected methionine (RPM). Thus, the source and quantity of dietary protein determine the availability of essential amino acids for animals (Ali *et al.*, 2009).

Addition of RPM to the diet of lambs increased the intake and digestibility of DM (Ali *et al.*, 2009; El-Tahawy *et al.*, 2015). This supplementation had beneficial effects on performance in the finishing period (Imik & Gunlu, 2011; El-Tahawy & Ismaeil, 2013; El-Tahawy *et al.*, 2015), which might be interpreted as indicating that methionine requirements are high in the last stage of growth (Abdelrahman *et al.*, 2003).

However, part of the supplemented methionine can be converted to choline (Pinotti *et al.*, 2002; NRC, 2007). In most forms, dietary choline is readily degraded in the rumen (Baldi & Pinotti, 2006). But sources of ruminally protected choline (RPC) are rarely included in the diet of lambs. Although the requirements for choline have not been established (NRC, 2007), there is evidence that finishing lambs respond to the addition of RPC (Bryant *et al.*, 1999; Li *et al.*, 2015; Martínez *et al.*, 2019). Because methylation needs may be so high that methionine is used to provide methyl groups, this may lead to methionine deficiency, thus reducing its availability for protein synthesis and limiting animal growth.

Organic farming is restricting the use of synthetic compounds such as chloride choline, which is used in the manufacture of RPC or ruminally protected synthetic amino acids. Therefore, natural alternative supplements that could replace synthetic products at a low cost while maintaining productive performance have been evaluated in rations for ruminants (Gutiérrez *et al.*, 2019; Martínez *et al.*, 2019; Mendoza *et al.*, 2019). Because choline and methionine are interrelated owing to the methylation process (Pinotti *et al.*, 2002; Martinov *et al.*, 2010) and because of the importance of methionine as a limiting amino acid for lamb

growth (NRC, 2007), the objective of this trial was to evaluate the effect of supplemented methionine and choline from polyherbal mixtures in finishing diets on productive performance and lipid metabolism of lambs.

## Material and Methods

All experimental procedures were performed according to the recommendations by the International Guiding Principles for Biomedical Research Involving Animals (CIOMS, 2012) and the Standards For Ethics, Biosafety and Animal Wellbeing of the Colegio de Postgraduados, Mexico, according to the Official Mexican Standard NOM-062-ZOO, 1999 (SAGARPA, 2001). The experiment was conducted in the sheep facilities at the experimental farm at the Colegio de Postgraduados, Montecillo, State of Mexico (98° 48' 27" W; 19° 48' 23" N). The climate is temperate and semi-arid, with an average annual temperature of 15.9 °C, infrequent frosts, 686 mm average annual rainfall, and an average altitude of 2241 m (García, 2004).

Forty Hampshire x Suffolk lambs weighing  $26.9 \pm 2.8$  kg were used for a 45-day experiment. The lambs were housed in individual cages equipped with single feeders and nipple drinkers. Lambs were dewormed (Chlorantel, 20 mg/kg bodyweight (BW) orally) and vaccinated against *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordelli*, *Clostridium perfringens*, *Pasterella multocida* type A, *Pasterella multocida* type D and *Pasterella haemolytica* (Bobact 8, 2.0 mL/animal). Feed was offered at 08h00 and 15h00. Water and feed were provided ad libitum. Lambs had eight days to adapt to the diets.

The lambs were allocated to one of four treatments, which were arranged as a 2x2 factorial, in which the factors were dietary supplementation with herbal methionine and choline at two levels (0% and 0.4% of DM). OptiMethione® and BioCholine Powder® were provided by Nuproxa Mexico (Querétaro, México). BioCholine is a polyherbal mixture, which contains *Achyranthes aspera*, *Trachyspermum ammi*, and *Andrographis paniculata*, whereas OptiMethione contains *Trigonella foenumgraecum* and *Allium sativa*. Natural choline conjugates were analysed by high-performance thin-layer chromatography (HPTLC) with lecithin as standard, and methionine was determined with HPLC (high-performance liquid chromatography) in the certified laboratory of Indian Herbs Specialities Pvt. Ltd. India. The BioCholine contained 1.6% of the natural conjugated form of choline such as phosphatidylcholine and equivalents, and OptiMethione contained 2.8% methionine. In this experiment, synthetic lysine was added to the diet to maintain an adequate balance between the amino acids, that is, lysine to methionine ratio. The experimental diets were formulated for a daily gain of 300 g/d (NRC, 2007) (Table 1).

The crude protein in the diets was determined with the macro Kjeldahl method (AOAC, 2005). Calcium and phosphorus were assessed by atomic absorption spectrophotometry (Karl *et al.*, 1979) with a Perkin Elmer 4000 Model (Series Lambda 2, Perkin Elmer Inc., Norwalk, CT, USA). Acid detergent fibre was estimated with the Van Soest *et al.* (1991) procedure.

Average daily feed intake (ADFI), average daily gain (ADG), feed to gain ratio (FGR), and final body weight (FBW) were recorded. Back fat thickness and chop area were measured with a real-time ultrasound (Silva, 2005) Sonovet 600 (Medison, Inc., Cypress, California, USA) with a 7.5 MHz transducer between the 12th and 13th ribs, on days 1 and 45 of the experiment. On the last day of the experiment, blood samples (5 mL) (preprandial at 08h00) were collected from the jugular vein by puncturing it with vacutainer tubes without anticoagulant (BD vacutainer), and immediately refrigerated at 4 °C. The blood samples were centrifuged (Sigma 2-16 k, Germany) at 3500 g for 15 minutes to obtain blood serum, which was stored in Eppendorf tubes and stored in a freezer (Sanyo MDF-436, USA) at -20 °C until analysis. In each sample, the concentration of total cholesterol (enzymatic oxidase-peroxidase method), glucose (enzymatic method), total protein (Biuret method), albumin (green bromocresol method), and high- and low-density lipoproteins (enzymatic method) were determined with kits from Spinreact (Barcelona, Spain).

Data were analysed as a completely randomized design with a 2x2 factorial arrangement, with four treatments and ten replicates, taking each lamb as an experimental unit. Shapiro-Wilk and Levene's tests were used to check for normality and homogeneity of variance. Data were analysed with the GLM procedure and Tukey's test ( $P \leq 0.05$ ) was used to compare treatment means (SAS Institute Inc., Cary, North Carolina, USA). Initial bodyweight was used as a covariate.

**Table 1** Experimental diets for lambs fed polyherbal mixtures with choline and methionine

Ingredient (%)	Treatments			
	Control	Methionine	Choline	Methionine+ Choline
Corn grain	55.28	55.28	55.28	55.28
Soybean meal	23.13	22.73	22.73	22.33
Lysine <sup>1</sup>	1.10	1.10	1.10	1.10
Herbal methionine <sup>2</sup>	0.00	0.40	0.00	0.40
Herbal choline <sup>3</sup>	0.00	0.00	0.40	0.40
Cane molasses	4.00	4.00	4.00	4.00
Alfalfa hay	10.05	10.05	10.05	10.05
Oat straw	5.00	5.00	5.00	5.00
Calcium carbonate	0.24	0.24	0.24	0.24
Sodium chloride	0.20	0.20	0.20	0.20
Mineral premix <sup>4</sup>	1.00	1.00	1.00	1.00
Nutrient composition calculated (dry matter basis)				
Metabolizable energy (Mcal/kg)	2.90	2.90	2.90	2.90
Crude protein (%)	19.00	18.90	18.70	18.85
Ruminal undegradable protein (%)	7.52	7.52	7.52	7.52
Calcium (%)	0.50	0.50	0.50	0.50
Phosphorus (%)	0.46	0.46	0.46	0.46
Acid detergent fibre (%)	15.05	15.05	15.05	15.05
Determined nutrient composition (dry matter basis)				
Crude protein (%)	18.85	18.96	18.48	18.79
Calcium (%)	0.78	0.84	0.73	1.11
Phosphorus (%)	0.54	0.49	0.53	0.52
Acid detergent fibre (%)	14.15	14.26	14.70	14.54

<sup>1</sup>Ajipro-L Ajinomoto 40% L-lysine, <sup>2</sup>Optimehion, <sup>3</sup>Biocholine, <sup>4</sup>Mineral premix: phosphorus 17.5%, calcium 6.5%, sodium 6.5%, sulphur 2%

## Results and Discussion

None of the productive variables and carcass characteristics was affected by dietary supplementation of RPC, RPM and in combination ( $P > 0.05$ ) (Table 2). It is possible that polyherbal methionine had little impact on the flow of estimated duodenal methionine (1%) and, because of the types of ingredients incorporated in the ration, the synthesis of microbial protein allowed all rations to supply adequate methionine (17 g/d). In addition, the diets were high in protein (19%). In studies in which responses to RPM were reported, the dietary crude protein concentration was lower than 15.7% (Abdelrahman *et al.*, 2003; Imik & Gunlu, 2011; El-Tahawy *et al.*, 2015). Moreover, Obeidat *et al.* (2008) suggested that methionine supplementation is not likely to produce beneficial effects on nutrient digestibility, performance and carcass characteristics of lambs fed with a high-performance diet. Rodríguez-Guerrero *et al.* (2018) also found that the addition of herbal RPM had no effects on bodyweight, DM intake and digestibility. They explained that the lack of response in lamb growth with supplemental RPM might have been caused by the low lysine content in the basal diet, which resulted in an inadequate ratio of lysine to methionine. In this work the authors assumed that the increased level of lysine in the diet would improve the balance between lysine and methionine. However, the supplementation regimes did not produce a beneficial response.

**Table 2** Effect of polyherbal choline and methionine on lamb performance and carcass characteristics

Performance measure	Treatment				SEM	P-value
	Control	Methionine	Choline	Methionine + choline		
Initial BW, kg	27.00	27.03	26.88	26.54	0.474	0.94
Final BW, kg	43.47	43.88	43.15	43.47	0.641	0.98
ADG, kg/d	0.343	0.351	0.338	0.345	0.009	0.97
DM intake, kg/d	1.503	1.532	1.559	1.471	0.026	0.81
Feed: gain ratio	4.47	4.46	4.59	4.46	0.094	0.94
Back fat, mm	3.55	3.77	3.66	3.37	0.093	0.56
Initial chop area, mm <sup>2</sup>	775	748	721	721	6.92	0.97
Final chop area, mm <sup>2</sup>	1141	1154	1137	1170	17.7	0.97

ADG: average daily gain; BW: bodweight; DM intake: dry matter intake

For the response to a ruminally protected amino acid to be expressed, lambs fed the basal diet must have limited duodenal flow in relation to the requirement. In Imik and Gunlu (2011), RPM supplementation increased the ADG significantly, and the estimated flow of methionine in the control treatment was 2.4 g/d. Although several experiments in lambs indicated that the response was positive when incorporating RPM in 0.20% to 0.33% of the diet (Imik & Gunlu, 2011; El-Tahawy & Ismaeil, 2013; El-Tahawy *et al.*, 2015), supplementation at up to 0.4% may have null or negative effects (Obeidat *et al.*, 2008; Ali *et al.*, 2009). Therefore, the methionine flux from a basal ration should be estimated and not generalized by dietary concentration. In addition to the response to RPM being quadratic, Abdelrahman and Hunaiti (2008) found that supplementation with 2 g/d RPM produced the greatest effects on ADG, FGR and carcass yield when they evaluated doses of 0 to 4 g/d.

The amount and quality of amino acids that reach the small intestine are influenced by the synthesis of microbial protein and the quantity of ruminally undegradable protein provided in the diet (Gao *et al.*, 2015). Thus, the types and levels of dietary protein have important effects on metabolizable essential amino acids (Ali *et al.*, 2009). Ruminally undegradable protein in this experiment was 7% of DM, which is considered a high concentration (NRC, 2007). Therefore, no methionine deficiency was shown, as occurs in diets with high ruminally degradable protein sources (Nolte *et al.*, 2004). Further, fermentable carbohydrates were able to provide carbon skeletons for the synthesis of microbial protein (Lapierre *et al.*, 2009) and allowed for adequate expression of growth by the lambs (El-Tahawy & Ismaeil, 2013).

Polyherbal choline intake (approximately 6 g/d) had no effect on the productive variables and carcass characteristics. Similar results were observed by Godinez-Cruz *et al.* (2015) and Rodriguez-Guerrero *et al.* (2018) with 4 g/d. However, Martinez *et al.* (2019) found that supplementation with approximately 5, 10 and 15 g/d of herbal RPC produced linear responses in daily gain, FBW, feed conversion, blood glucose, and blood phosphatidylcholine as the level of dietary herbal choline increased. Concurrently, total protein, globulins, and low-density lipoproteins showed a quadratic response. There was also evidence that the inclusion of plant feed additives containing conjugates of choline improved milk yield, fertility and important health indicators in first-lactation cows, which helped to lower the replacement rate (Gutierrez *et al.*, 2019). Li *et al.* (2015) found production traits and meat quality exhibited quadratic responses to supplementation with RPC, with the best response being with 0.25% RPC in the lamb diets and with negative effects at 0.75% on ADG (representing 2.6 and 7.8 g/d of RPC, respectively). Bryant *et al.* (1999) observed a 10% increase in ADG by adding 0.25% choline to the diet (equivalent to 2 g/d of RPC). However, there was no difference among other levels (0, 0.5, and 1.0%) of RPC. The degree of protection of commercial RPC products is not known precisely and varies in choline content (Kung *et al.*, 2003; Jayaprakash *et al.*, 2016). In addition, the physiological needs for methylation may be covered by methionine (Pinotti *et al.*, 2002; Martinov *et al.*, 2010), thus making it difficult to establish a recommendation for RPC and herbal products, yet performance data indicated (Martínez-Aispuro *et al.*, 2019) that it should not exceed a daily dose of 4 g/d of RPC.

In spite of the metabolic relationship of choline and methionine as donors of methyl groups, no advantage was found in supplementing these nutrients together. It has been suggested that methionine may spare choline utilization in dairy cattle (Soltan *et al.*, 2012) and dairy sheep (Tsiplakou *et al.*, 2017). Supplementation with herbal choline plus herbal methionine improved milk production and 4% fat-corrected milk in supplemented cows (Mendoza *et al.*, 2019). However, the positive response to the supplemented

combination of choline and methionine in diets for dairy ewes may be present only when conditions of high-nutrient demands and low-protein diets are combined (Piepenbrink & Overton, 2003).

The addition of choline and methionine to the diet had no detectable effect on the amount of subcutaneous fat in the chop area, which is consistent with other experiments with levels of 0.25 - 0.75% RPC (Bryant *et al.*, 1999; Li *et al.*, 2015). However, in lactating sheep, the combination of choline and methionine increased the amount of fat in milk (Tsiplakou *et al.*, 2017). Previous work showed the lipotropic effect of both nutrients in dairy cattle (Piepenbrink & Overton, 2003; Osorio *et al.*, 2014). Several mechanisms of action mediated by carnitine synthesis have been discussed (Bryant *et al.*, 1999) and their effects on the deposition of fat (Eklund *et al.*, 2005).

In the present experiment, dietary supplementation with RPM and RPC did not affect the concentrations of cholesterol, high-density lipoproteins, glucose, proteins and globulins in blood plasma ( $P > 0.05$ ) (Table 3). Only the concentration of albumins ( $P < 0.004$ ) was increased by combining RPM and RPC (Table 3). Other studies in sheep showed that the inclusion of RPC at levels of 0.25 - 0.75% did not modify the concentration of cholesterol and triglycerides in plasma (Bryant *et al.*, 1999; Baldi & Pinotti, 2006). However, Imik and Gunlu (2011) found that adding 0.20% of protected methionine to the diet for fattening lambs increased the concentration of triglycerides and reduced the concentration of glucose.

**Table 3** Effect of polyherbal mixtures with choline and methionine on lamb blood metabolites

Serum constituent	Treatment				SEM	P -value
	Control	Methionine	Choline	Methionine + choline		
Cholesterol, mg/dL	54.30	49.78	55.62	61.24	2.118	0.28
HDL, mg/dL	28.35	24.41	31.92	35.57	1.814	0.15
LDL, mg/dL	19.35	25.10	17.92	17.99	1.165	0.34
Glucose, mg/dL	83.76	85.85	88.70	89.56	1.779	0.66
Protein, g/dL	5.84	6.10	6.46	6.30	0.123	0.34
Albumins, g/dL	3.02 <sup>ab</sup>	2.71 <sup>b</sup>	2.88 <sup>b</sup>	3.25 <sup>a</sup>	0.057	0.004
Globulins, g/dL	2.83	3.39	3.57	3.06	0.083	0.14

HDL: high-density lipoproteins; LDL: low-density lipoproteins

Although the requirements of choline and methionine for lambs have not been well established (NRC, 2007), the conditions in which their supplementation is required must be identified to potentiate the productive performance in relation to the demands for methylation and for microbial protein synthesis and the interrelation of these metabolites in the organism.

## Conclusion

The inclusion of polyherbal mixtures containing either methionine or choline or in combination, at a concentration of 0.4% DM, in finishing lamb diets provided limited if any benefit in productive response or serum metabolites indicative of lipid metabolism.

## Authors' Contributions

JRBG, JAMA, GDMM, JLCM, TSTE, JLFV, MAAM were responsible for the design and execution of the project. All co-authors participated in management and discussion of the results, statistical analysis and writing of the manuscript.

## Conflict of Interest Declaration

Authors declare that there is no conflict of interest regarding the publication of this article.

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