Chilean J. Agric. Anim. Sci., ex Agro-Ciencia (2024) 40(2): 353-361.
https://doi.org/10.29393/CHJAAS40-30ERAO50030

ISSN 0719-3890 online

EVALUATION OF HERBAL CHOLINE AND CHELATED MINERALS ON GROWTH AND RUMINAL BACTERIA IN KATAHDIN RAMS

Alejandro Ley-de Coss[1], Cándido Enrique Guerra-Medina[2,3], Miguel Chávez-Espinoza[3a], Edgar Meraz-Romero[4], and Oziel Dante Montañez-Valdez[3b*]

[1] Universidad Autónoma de Chiapas. Facultad de Ciencias Agronómicas; Campus V. Villaflores, C. P. 30470, Chiapas, Mexico
https://orcid.org/0000-0001-5982-300X

[2] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Centro de Investigación del Pacifico Sur. Rosario Izapa, Tuxtla Chico, C. P. 30890 Chiapas, Mexico
https://orcid.org/0000-0001-9943-0032

[3a] Grupo de Investigación en Nutrición Animal (GINA). Universidad de Guadalajara, Centro Universitario del Sur. Ciudad Guzmán, C. P. 49000 Jalisco, Mexico
https://orcid.org/0000-0001-7293-3908

[3b] Grupo de Investigación en Nutrición Animal (GINA). Universidad de Guadalajara, Centro Universitario del Sur. Ciudad Guzmán, C. P. 49000 Jalisco, Mexico
https://orcid.org/0000-0001-9539-6623

[4] Universidad Nacional Autónoma de México. Facultad de Medicina Veterinaria y Zootecnia. Centro de Enseñanza, Investigación y Extensión en Producción Animal en Altiplano. Tequisquiapan, C. P. 76790 Querétaro, Mexico
https://orcid.org/0000-0002-6311-8327

* Corresponding author: montanez77@hotmail.com

ABSTRACT

This study evaluated the effect of herbal choline (Ch) and chelated minerals (CM), alone or combined, on growth and rumen bacteria in rams. Four entire male Katahdin sheep with initial live weight of 25 ± 0.5 kg were randomly distributed in a 4 × 4 Latin square, where each period consisted of 21 d (7 d of adaptation to the diet and 14 d for sample collection). Rams were housed in metabolic cages and fed a basal diet (BD) for growth containing 1.17 Mcal of NEG and 14% CP. The treatments evaluated were: T1) Basal diet (Control, BD); T2) BD + 4 g herbal choline (Ch); T3) BD + 1 g of chelated minerals (CM); and T4) BD + 4 g of Ch + 1 g of CM (BD+Ch+CM). The treatments Ch alone or in combination with CM improved daily weight gain ($p < 0.05$), without showing changes in dry matter intake, ruminal pH, or in total, cellulolytic and lactic acid bacterial populations ($p > 0.05$). The addition of choline alone or in combination with chelated minerals improves daily weight gain, without altering the pH or ruminal bacteria in rams.

Keywords: Rumen microorganism, plant extract, minerals, ruminants, body gain.

Received: February 27, 2024 Accepted: August 12, 2024

INTRODUCTION

According to the animal genetic improvement, nutritional requirements have increased. Specifically, the choline requirement is greater than that provided by the ingredients used in the formulation of the diet or by the synthesis of the microorganisms inhabiting the rumen (Mendoza et al., 2019). The use of rumen-protected choline (RPC) has shown that it is a limiting nutrient in milk and beef cattle production (Zhou et al., 2016). As a B complex vitamin, choline is an essential nutrient for the optimal development of the animal due its activity in metabolic pathways and the use of metabolite forms (phosphatidylcholine, lysophosphatidylcholine and sphingomyelin), which are constituents of cell membranes and have an important function in lipid metabolism and cell signalling (Jian et al., 2014).

The evaluation of herbal products in ruminant production has shown that choline is an alternative to improve meat production in sheep (Jin et al., 2023), goats (Mendoza et al., 2019), steers and milk production in dairy cows (Holdorf et al., 2023; Martínez et al., 2021). Previous studies have shown that RPC supplementation in ruminants increased choline availability, which in turn improved milk production, availability and fat content, while it also increased protein content in milk and daily weight gain in sheep at doses of up to 2.5 g kg^{-1} DM of RPC in the diet (Huang et al., 2023; Li et al., 2015).

Products based on herbal choline (Ch) available in the market are polyherbal mixtures with different effects on animal health, including antioxidant, antiparasitic, nephroprotective, antimicrobial, anti-inflammatory properties, also being a source of betaine (Tatke et al., 2014; Okhuarobo et al., 2014) or important sources of choline in the form of phosphatidylcholine and phosphatidylserine (Baldissera et al., 2019). It has been reported that the combination of RPC and methionine, which is not degradable in the rumen, could function as bioactive compounds that help prevent and protect ruminants from diseases, mainly in the peripartum (Zhou et al., 2016). Polyherbal mixtures of choline and methionine have been evaluated and classified as nutrients with nutraceutical properties (Mendoza et al., 2019). As the RPC, the chelated minerals pass through the rumen and get absorbed intact post-ruminally in the intestine; these chelates accomplishing blood plasma are intact and released at the site of utilization (Gayathri and Panda, 2018). In this study, it was hypothesized that supplementation of choline and chelated minerals improves productive performance without having negative effects on the environment and ruminal microorganisms in ewes.

Therefore, this study aimed to determine the effect of the addition of choline and chelated minerals on dry matter intake, daily weight gain, feed efficiency, and concentrations of total, cellulolytic and lactic acid bacteria, as well as on rumen pH in Katahdin rams.

MATERIALS AND METHODS

Study site. The experiment was carried out in the Agricultural Health Laboratory of the University Center for Technology Transfer (CUTT) “San Ramón” of the Faculty of Agricultural Sciences; Campus V of the Autonomous University of Chiapas, Villaflores, Chiapas (16°27′59″ LN and 93°28′43″ WL). The predominant climate is warm subhumid (AW1) (W)(i´) g, with an average annual rainfall of 1200 mm distributed from the months of June to November, with an average temperature of 22 °C and an altitude of 591 m (INEGI, 2023).

Animals and treatments. Four entire male Katahdin sheep with initial live weight of 25 ± 0.5 kg were randomly distributed in a 4 × 4 Latin square, with four treatments and four experimental periods (repetitions). Each experimental period consisted of 21 d, 7 d for adaptation of the animals to the diet and 14 d for sample collection. The appropriate ethical review committee approval was obtained. The authors confirm that they followed the standards of the Universidad Autónoma de Chiapas for the protection of animals used for scientific purposes, in accordance with the Mexican official norm of technical specifications for the production, care, and use of laboratory animals (NOM-062-ZOO-1999). The rams were housed in individual metabolic cages of 1.2 m^2, provided with automatic waterers and metal feeders. Each of them was dewormed externally with Fipronil 2% (1 mg kg^{-1} percutaneous BW) and internally with oral Albendazole (5 mg kg^{-1} BW).

Experimental diets. A basal diet (BD) containing 1.170 Mcal of NEG and 14% of CP was formulated, considering the nutritional requirements of the NRC (2007), for a weight gain of up to 200 g per day. The chemical composition of this diet was subsequently determined using the AOAC (2016) methods. The neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were determined according to the technique described by Van Soest (1991). The evaluated treatments were: T1) Basal diet (Control, BD); T2) BD + 4 g herbal choline (Ch); T3) BD + 1 g of chelated minerals (CM); and T4) BD + 4 g of Ch + 1 g of CM (BD+Ch+CM). The herbal choline doses of 4 g in the experimental diets were applied according at the manufacturer's recommendation of 4 – 6 g/animal/day for small ruminants of BioCholine® (Nuproxa, Etoy, Switzerland). BioCholine® is a commercial polyherbal mixture powder based of the plants *Achyranthes aspera*, *Azadirachta indica*, *Trachyspermum ammi*, *Citrullus colocynthis* and *Andrographis paniculate*. The chelated minerals (Biotecap®, Jalisco, Mexico) used are a mixture of minerals containing Se, 500 ppm; Zn, 2500 ppm; I, 30 ppm; Co, 30 ppm; Cr, 500 ppm; Cu, 200 ppm; Mn, 2500 ppm; and Fe, 1500 ppm. Both compounds were added directly to the diet and mixed manually to ensure a uniform distribution. The diets were packaged and stored at room temperature. The feed was offered at 08:00 and 16:00 h, in addition to providing freshwater *ad libitum* (Table 1).

Consumption and daily weight gain. After an experimental period of 21 d, the first 7 d were used for the adaptation of the animals to the experimental diets, recording individual daily consumption; daily dry matter intake (DMI, g d^{-1}) was estimated by difference between the feed offered and rejected by each animal per day. The rams were weighed at the beginning of the experiment and every week before morning feeding in order to calculate daily weight gain (DWG, g d^{-1}). The feed conversion (FC) was calculated by dividing the DMI by the DWG in each treatment and experimental period.

Rumen liquid. On the last day of each experimental period, two hours after the 8:00 a.m. feeding, 50 mL of fresh ruminal fluid was extracted with an oesophageal probe. The probe was composed of a metal probing head with several holes and a plastic tube of 150 cm long and 14 mm diameter, which ended in a plastic rod to facilitate the fluid collection and the cleaning. In each experimental period, the ruminal fluid extracted from each ram was filtered with sterile gauze to remove solid fractions of digesta. The pH was measured with a potentiometer (Orión A250, Orion Research, Inc. USA).

Concentration of total, cellulolytic and lactic acid bacteria. A culture medium was prepared to establish the concentration of total bacteria (TB) and cellulolytic bacteria (CB), using the technique described by Ley de Coss et al. (2013). The anaerobic culture medium contained: 0.06 g of D-(+)-glucose + 0.06 g D-cellobiose + 0.06 g starch (J. T. Baker, México, México), 30 mL of clarified ruminal fluid, 5.0 mL of mineral solution I [6 g K_2HPO_4 (Meyer, CdMex, MExico) in 1000 mL of H_2O], 5.0 mL of mineral solution II [6 g KH_2PO_4 (Meyer) + 6 g $(NH_4)_2SO_4$ (Meyer) + 12 g NaCl (Meyer) + 2.45 g $MgSO_4$ (Meyer) + 1.6 g $CaCl_2 \cdot \boldsymbol{H_2}O$ (Fermont, Monterrey, NL) in 1000 mL of H_2O], 2.0 mL of 8% Na_2CO_3 solution

Table 1. Ingredients and chemical composition of the experimental basal diet.

Ingredients	%
Maize meal	31.00
Soybean meal	20.00
Rumen by-pass fat*	2.00
Maize stover	39.00
Molasses	5.00
Salt	1.00
Mineral mix¥	2.00
Chemical composition	
Dry matter	68.60
Crude protein	14.00
Ether extract	4.80
Neutral detergent fiber	26.60
Acid detergent fiber	15.10
Calcium	0.90
Phosphorus	0.36
Net energy gain (Mcal kg^{-1})	1.17

*Palm oil (Palm fatty acid distillate, Palmalife®, Mexico).
¥Mineral mix: Ca, 20%; P, 3%; Mg, 6.5%; and S, 0.5%.

(Meyer), 2.0 mL of sulfide-cysteine solution [2.5 g L–cysteine (Sigma-Aldrich, México, México) in 15 mL of 2N NaOH (Meyer) + 2.5 g Na_2S-$9H_2O$ (Meyer) volumetric in 100 mL H_2O], 0.2 g peptone trypticase (MCD Lab) and 0.1 mL of 0.1% resazurin solution (Sigma-Aldrich). After measuring pH, the extracted rumen fluid was immediately taken to an incubator at 39 °C, to subsequently inoculate 0.5 mL of rumen fluid to the culture media in triplicate. For counting lactic acid bacteria (LAB), an enriched anaerobic MRS medium (Sigma®) was used. The amount of total, cellulolytic and lactic acid bacteria was determined by the most probable number technique (Harrigan and Cance, 1990).

Experimental design and statistical analysis. A 4 x 4 Latin square design was used with the following statistical model:

$$Y_{ijk} = \mu + H_i + C_j + T_k + \varepsilon_{ijk}$$

Where: Y_{ijk} = Response variable, μ = General mean, Hi = Effect of the *ith* period (row) C_j = Effect of the *jth* animal (column), T_k = Effect of the *kth* treatment (diet) εijk = Error experimental.

DMI, DWG, FC and ruminal pH data were analysed using PROC GLM from SAS (2011). The statistical model included the effect of the period, the animal, and the treatment. Each data set was tested for normality using Levene's test, while non-parametric analyses were used for data that differed significantly from a normal distribution (p < 0.05). For the variable total, cellulolytic and lactic acid bacteria, the same model (PROC GLM) was used using the Kruskal-Wallis test, with independent rank data (Wilcoxon). Means were compared using the Tukey's test. Significance was declared at a p-value ≤ 0.05.

RESULTS AND DISCUSION

Table 2 shows the response to the addition of choline and chelated minerals to the diet of rams on DMI, FC, and DWG. Treatments with additives (Ch and CM) had no effect on the final weight and DMI of the sheep (p >0.05), but better numerical values were observed in diets that combined both additives. The DWG increased in the treatment with the additives, with a better response in the diet added with Ch + CM (p <0.05), followed by the diet with Ch, and with less effect with the BD + CM or the control diet. This same result was found in the FC (p <0.05), where the mixture of both additives (Ch + CM) was superior compared to the other treatments.

The use of protected choline in ruminants improved the productive variables of the animals subjected to its consumption, even showing greater milk production and muscle synthesis during growth. In goats, a higher DMI has been observed when they received protected choline, reporting feed consumption of up to 1800 g animal^{-1} d^{-1}, and with an increase in milk production, as well as a higher weight in lambs at birth (Ardalan et al., 2011; El-Grendy et al., 2012; Suárez et al., 2023). Kawas et al. (2020) reported no significant differences in dry matter intake (DMI) and FC among supplementation doses of 0, 0.1, 0.2, and 0.3% RPC on dry-matter basis of feedlot Saint Croix lambs. In the present study, the DMI did not show a change due to the addition of Ch, CM or their combination, but there was an effect on DWG and FC due to the addition of Ch or its combination with CM. This effect is similar to what was found in goats fed with the addition of Ch in doses of 4 and 8 g animal^{-1} d^{-1} to the diet, showing improved DWG and milk production values (Supriyati et al., 2016).

At doses of 0.0, 5.0 and 7.5 g kg^{-1} of DM,

Table 2. Dry matter intake (DMI, g d^{-1}), daily weight gain (DWG, g d^{-1}) and feed conversion (FC) of Katahdin rams fed with experimental diets.

	Treatments[1]				
	BD	BD+Ch	BD+CM	BD+Ch+CM	SEM[7]
IW[2]	25.5	24.5	25.0	25.5	0.71
FW3	33.0	32.4	32.2	33.8	0.71
DWG[4]	311.6b	328.2ab	301.3b	347.0a	13.61
DMI[5]	1251.8	1233.8	1240.0	1209.8	36.01
FC[6]	4.0b	3.8ab	4.1b	3.5a	1.96

[1] BD = Basal diet, control; BD + Ch = Basal Diet + 4 g of herbal choline; BD + CM= Base diet + 1 g of chelated minerals; BD +Ch + CM= Basal diet + 4 g of Ch + 1 g of CM (BD+Ch+CM). [2] IW: Initial weight; [3] FW: Final weight; [4] DMI: dry matter intake; [5] DWG: daily weight gain. [6] FC: Feeding conversion; [7] SEM: standard error of the mean.

[a, b]: Values with different literals in the same row are different (p <0.05).

protected choline in the diet of rams reduced the DMI, DWG and feed conversion; however, a low DMI but a higher DWG were observed with the dose of 2.5% g kg^{-1} of DM (Li et al., 2015). The effect of RPC depends on bioavailability and rumen passing, not only on the dose. In this sense, Kawas et al. (2020) argued that this situation has not been elucidated in small ruminants. Methionine and choline are nutrients that promote the synthesis of phosphatidylcholine, increasing low-density lipoproteins (LDL) synthesized in ruminants, facilitating the absorption and transport of lipids to the liver.

In diets deficient in proteins, folates and amino acids (AA), particularly methionine and choline, have shown the potential for the synthesis of LDL due to a dependent effect on the profile of AA absorbed from the small intestine in ruminants. Therefore, it is important to add a source of the protein degradable in the rumen (PDR) and non-degradable in the rumen (PNDR) to diets, due to the effect they have on the concentration of ruminal microorganisms and the passage of microbial protein of high biological value towards the abomasum and the subsequent absorption of AA in the small intestine by animals (Ardalan et al., 2011). Choline is an essential nutrient for mammals. When there is an insufficient supply of methionine and folates in the diet, vitamin B12 also intervenes in this process. The dynamic interactions between these components introduced the concept of choline as a vitamin-like compound. Two types of choline functions are known: choline *per se*, for which the choline moiety is required; and methyl donor. Choline alone plays an important role in lipid metabolism, particularly in lipid transport, as a lipotropic agent. Choline is also an important source of labile methyl groups for the biosynthesis of other methylated compounds. Based on this second function, choline and methionine are interchangeable as sources of methyl groups. Therefore, choline occupies a key position between energy and protein metabolism in mammals (Ardalan et al., 2011; Suárez et al., 2023).

However, the metabolism of choline and the methyl group in ruminants is different. In ruminant adults, choline is extensively degraded in the rumen; dietary choline contributes insignificantly to the body choline reserve; and the metabolism of methyl groups is generally conservative with a relatively low rate of methyl catabolism and a high rate of de novo synthesis of methyl groups to the tetrahydrofolate (THF) system. This may be exacerbated in lactating dairy ruminants, in which dietary availability of choline is almost non-existent, but production of methylated compounds in milk is high, while methionine and other sources of methyl groups are likely to be scarce, especially at the beginning of breastfeeding. Considering this, the hypothesis that choline may be a limiting nutrient for milk production has been formulated and tested in several studies (Supriyati et al., 2016; Suárez et al., 2023).

Martinez et al. (2019) reported that herbal choline can be included at 6 g kg^{-1} of DM to improve male Hampshire × Suffolk lambs performance in finishing diets by the presence of phosphatidylcholine in blood, but argued that levels of 9 g Kg^{-1} of DM could affect ruminal fermentation due the presence of other metabolites. This effect was observed with the highest DWG in the Ch and CM treatments, with no effects on the total bacterial population in the rumen of all animals in all treatments. On the other hand, Rodríguez et al. (2018) reported that supplementation with RPC and biocholine did not improve the performance of lambs in the growth phase. However, in both additives, beneficial effects were observed on hepatic metabolism with higher blood levels of non-esterified fatty acids, glucose and cholesterol compared to control animals, demonstrating that there is lipid mobilization of Pelibuey x East Friesian lambs in growth phase.

The effect of the additives in the ram diets on the pH and concentration of ruminal bacteria is presented in Table 3. The concentration of total, cellulolytic and lactic acid bacteria did not change (p >0.05) due to the treatments used in this study. Concentrations of TB and LAB were higher in the diet with Ch + CM, but lower in terms of CB. Regarding rumen pH, there were no significant differences between treatments (p >0.05).

Several studies have evaluated the use of herbal mixtures in dairy cattle (Gutierrez et al., 2019; Imaz et al., 2022), where photogenic extracts and their secondary metabolites have been shown to possess antimicrobial properties that stimulate the immune system (Upadhaya and Kim, 2017). Mendoza et al. (2019) argued that the presence of volatile metabolites, such as 2-Undecenal, 8-p-menthane diamine, 4-vinylguaiacol, β-pinene and p-cresol, have bacteriostatic and bactericidal effects. In the present study, however, there was no reduction in the concentration of the groups of ruminal bacteria analysed (BT, CB and LAB), while an increase in the concentration of LAB was observed in the treatments that combined both additives. In addition, the effect of Ch in combination with CM on the concentrations of TB, CB and LAB in the rumen of fattening rams was homogeneous in all treatments, and thus there were no significant changes in these bacterial

Table 3. Effect of herbal choline and chelated minerals on the pH and concentration of ruminal bacteria.

	Treatments[1]				
	BD	BD + Ch	BD + CM	BD + Ch + CM	SEM[5]
TB[2] ×10[12]	1.43	2.59	2.21	3.56	2.51
CB[3] ×10[8]	25.00	25.50	27.50	23.50	6.51
LAB[4] ×10[9]	2.17	2.00	1.75	2.64	2.14
Ruminal pH	6.65	6.55	6.65	6.62	0.20

[1] DB = Basal diet, control; BD + Ch = Basal diet + 4 g of herbal choline; BD + CM= Basal diet + 1 g de chelated minerals; BD +Ch + CM= Basal Diet + 4 g de Ch + 1 g de CM (BD+Ch+CM).
[2] TB: Total bacteria; [3] CB: Cellulolytic bacteria; [4] LAB: Lactic acid bacteria; [5] SEM: Standard error of the mean.
The values are similar between treatments (p >0.05).

groups. The concentrations of TB, CB and LAB are within the normal concentrations reported in the rumen. These findings highlight the importance of considering dietary composition as a crucial factor in the modulation of ruminal microbiota groups.

Arce-Cordero et al. (2022) reported that unprotected choline chloride in diets with 30% NDF particularly decreases the abundance of bacterial involved in fiber degradation and increases abundance of bacteria mostly involved in degradation of non-structural carbohydrates and propionate synthesis. Diets low in NDF reduce the pH of the rumen, decreasing the metabolic efficiency of bacteria in the synthesis of microbial protein. Therefore, it is necessary to ensure adequate levels of PDR and PNDR, which increase ammonia (NH_3) content and the microbial protein synthesis, having an impact on the production levels of those animals to which Ch is added to the diet. Guo et al. (2020) found no significant differences with the supplementation of RPC on ruminal pH in pregnant ewes suffering from negative energy balance. Increased microbial protein synthesis reduces the demand for essential AA, thereby diluting the potential effects of protected choline. Furthermore, high levels of NH_3 help regulate rumen pH, with the consequence of increasing the cost of ATP for the biosynthesis and excretion of urea by the liver and kidney (Ardalan et al., 2011; Holdorf et al., 2023).

In the present study, the combination of Ch and CM showed the best values, probably due to a greater flow of AA such as histidine (Lee et al., 2012), which improves the productive response of the animal due to greater concentration and bacteria development in the rumen of the animal. In fact, the synergy between the metabolism of methionine and choline was observed. Methionine is used for the synthesis of choline, and thus it is necessary to cover the CP requirements in the diets of the treated animals as carried out in this study (CP, 14%), mainly for the sheep growth and development stages.

It is important to consider that herbal additives are mixtures of plants or specific plant parts containing the highest concentration of biologically active molecules that have an effect on ruminal fermentation as well as a positive impact on production (Kolling et al., 2018). For instance, herbal mixtures with phosphatidylcholine in synergy with methionine have been shown to improve health and production of dairy cows (Mendoza et al., 2019; Gutiérrez et al., 2019). In addition, natural products have antioxidant activity that allows the prevention and treatment of diseases, inhibition of bacterial growth, modulation of metabolic pathways and an improvement in animal health and productive development (Kholif and Olafadehan, 2021). Furthermore, it has been reported that the microbial population of the rumen degrades the choline added to the diet, which implies increasing is dose. However, the use of protected choline in dairy cattle has shown a positive effect on milk production (de Veth et al., 2016; Liu et al., 2019). In beef cattle and goats, the addition of rumen non-degradable choline chloride at lower dietary concentrations has improved feed conversion and DWG (Habeeb et al., 2017). RPC probably protected liver function by transporting non-esterified fatty acids (NEFA) out of the liver and utilized by other tissues (Guo et al., 2020), while Cu and Zn sulphates, as chelated minerals, improved liver function. Lambs supplemented with Cu and Zn-Met reflected higher concentrations of Cu and Zn in serum and wool, and thus are easily absorbed through the intestinal mucosa and transported intact (Shinde et al., 2013).

CONCLUSIONS

The inclusion of herbal choline combined with chelated minerals in the diet of rams improves feed conversion and daily weight gain, without affecting dry matter intake and the stability of the ruminal bacteria. The herbal choline (BioCholine®) under this study showed potential as alternative feed resource to improve feeding conversion ratio in fattening hair sheep in southeastern Mexico.

ACKNOWLEDGMENTS

To Yajeiri Natali José Velázquez and Josué Figueroa Mendoza, students of the Bachelor's Degree in Agricultural Engineering at the Faculty of Agricultural Sciences. To students of the Faculty of Agricultural Sciences; UNACH Campus V that collaborated and the technical person responsible for project 06/AGV/RPR/010/19, titled: Biotechnological strategy to stimulate rumen development in suckling calves. To Dr. José Ricardo Bárcena Gama of the Livestock Program of the Montecillo Campus of Postgraduate College for the donation of the herbal product to carry out this research work.

Authors' contribution

All authors of the article had an active participation in: bibliographic review, in the development of the methodology, in the discussion of the results, and review and approval of the final version of the article.

LITERATURE CITED

AOAC. 2016. Association of Official Analytical Chemists. Official Methods of Analysis of Analysis (20th) Rockville, MaryLand, USA: AOAC International.

Arce-Cordero, J. A., P. Fan, H.F. Monteiro, X. Dai, K.C. Jeong, and A.P. Faciola. 2022. Effects of choline chloride on the ruminal microbiome at 2 dietary neutral detergent fiber concentrations in continuous culture. Journal of Dairy Science 105(5):4128-4143. https://doi.org/10.3168/jds.2021-21591

Ardalan, M., V. Dehghan, K. Rezayazdi, and N. G. Hossein. 2011. The effect of rumen-protected methionine and choline on plasma metabolites of Holstein dairy cows. Journal of Agriculture Science 149 (5): 639–646. https://doi.org/10.1017/S0021859610001292

Baldissera, M.D., C.F. Souza, B. Baldisserotto, F. Zimmer, D. Paiano, T.G. Petrolli, and A. S. Da Silva. 2019. Vegetable choline improves growth performance, energetic metabolism, and antioxidant capacity of fingerling Nile tilapia (*Oreochromis niloticus*). Aquaculture 501: 224-229. https://doi.org/10.1016/j.aquaculture.2018.11.021

de Veth, M.J., M. A. Artegoitia, S.R. Campagna, H. Lapierre, F. Harte, and C.L. Girard. 2016. Choline absorption and evaluation of bioavailability markers when supplementing choline to lactating dairy cows. Journal of Dairy Science 99(12):1–13. http://dx.doi.org/10.3168/jds.2016-11382

El-Gendy, M.E., K.F. El-Riedy, H.S. Sakr, and H.M. Gaafar. 2012. Effect of rumen protected methionine and/or choline additives on productive performance of Zaraibi goats. Nature and Science 10(10):35-41. http://www.sciencepub.net/nature/ns1010/006_10162ns1010_35_41.pdf

Gayathri, S. L., and N. Panda. 2018. Chelated minerals and its effect on animal production: A review. Agricultural Reviews 39(4): 314-320. https://doi:10.18805/ag.R-1823

Guo, C., Y. Xue, Y. Yin, D. Sun, H. Xuan, J. Liu, and S. Mao. 2020. The effect of glycerol or rumen-protected choline chloride on rumen fermentation and blood metabolome in pregnant ewes suffering from negative energy balance. Animal Feed Science and Technology 268: 114594. https://doi.org/10.1016/j.anifeedsci.2020.114594

Gutiérrez, A.R., C. Sánchez, and D. G. Mendoza. 2019. Effect of including herbal choline in the diet of a dairy herd; a multiyear evaluation. Emirate Journal of Food Agriculture 31(6):477-481. https://doi:10.9755/ejfa.2019.v31.i6.1971

Habeeb, A.A.M., A. E. Gad, M. A. A. Atta, and M.A.M Abdel-Hafez. 2017. Evaluation of rumen-protected choline additive to diet on productive performance of male Zaraibi growing goats during hot summer season in Egypt. Tropical Animal Health and Production 49:1107-1115. https://doi.org/10.1007/s11250-017-1292-x

Harrigan, W.F., and E. Mc. Cance. 1990. Laboratory Methods. In W. F. Harrigan (ed.). Food and Dairy Microbiology. 8th Ed. Academic Press. Great Britain.

Holdorf, H.T., W. E. Brown, G. J. Combs, S. J. Henisz, S.J. Kendall, M.J. Caputo, K. E. Ruh, and H. M. White. 2023. Increasing the prepartum dose of rumen-protected choline: Effects of maternal choline supplementation on growth, feed efficiency, and metabolism in Holstein and Holstein × Angus calves. Journal of Dairy Science 106(9):6005-6027. https://doi.org/10.3168/jds.2022-23068

Huang, B., M. Z. Khan, X. Kou, Y. Chen, H. Liang, Q. Ullah, N. Khan, A. Khan, W. Chai, and C. Wang. 2023. Enhancing metabolism and milk production performance in periparturient dairy cattle through rumen-protected methionine and choline supplementation. Metabolites 13(10):1080. https://doi.org/10.3390/metabo13101080

Imaz, J.A., S. García, and L.A. González. 2022. The metabolomics profile of growth rate in grazing beef cattle. Scientific Reports 12:2554. https://doi.org/10.1038/s41598-022-06592-y

INEGI. 2023. Instituto Nacional de Estadística y Geografía. Dirección General de Geografía y Medio Ambiente. Catálogo Único de Claves de Áreas Geoestadísticas Estatales, Municipales y Localidades. México. Disponible en http://www.inegi.org.mx/geo/contenidos/geoestadistica/catalogoclaves.aspx (Available February 27, 2024).

Jiang, X., J. Yan, and M.A. Caudill. 2014. Choline. p 491-513. In J. Zempleni, J.W. Suttie, J.F. Gregory and P.J. Stover (eds.) Handbook of Vitamins (5th Edition). CRC Press, Florida, USA. https://doi.org/10.1201/b15413

Jin, Y., H. Li and H. Wang. 2023. Dietary rumen-protected choline supplementation regulates blood biochemical profiles and urinary metabolome and improves growth performance of growing lambs. Animal Biotechnology 34(3):563-573. https://doi.org/10.1080/10495398.2021.1984247

Kawas, J.R., J.F. García-Mazcorro, H. Fimbres-Durazo, and M.E. Ortega-Cerrilla. 2020. Effects of rumen-protected choline on growth performance, carcass characteristics and blood lipid metabolites of feedlot lambs. Animals 10(9): 1580. https://doi.org/10.3390/ani10091580

Kholif, A.E., and O. A. Olafadehan. 2021. Essential oils and phytogenic feed additives in ruminant diet: Chemistry, ruminal microbiota and fermentation, feed utilization and productive performance. Phytochemistry Reviews 20(6):1087-1108. https://doi.org/10.1007/s11101-021-09739-3

Kolling, G.J., S.C.B Stivanin, A.M Gabbi, F.S Machado, A.L. Ferreira, M. M. Campos, T.R. Tomich, C.S. Cunha, S.W. Dill, L.G.R. Pereira, and V. Fischer. 2018. Performance and methano emissions in dairy cows fed oregano and green tea extracts as feed additives. Journal of Dairy Science 101(5): 4221-4234. https://doi.org/10.3168/jds.2017-13841

Lee C, A. N. Hristov, T.W. Cassidy, K.S. Herley, H. Lapierre, G. A. Vargas, M. J. de Veth, R.A. Patton, and C. Parys. 2012. Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein deficient diet. Journal of Dairy Science 95(10): 6042-6056. http://dx.doi.org/10.3168/jds.2012-5581

Ley de Coss, A. C. Arce-Espino, M. Cobos-Peralta, D. Hernández-Sánchez y R. Pinto-Ruiz. 2013. Estudio comparativo entre la cepa de *Pediococcus acidilactici* aislada del rumen de borregos y un consorcio de bacteria ruminales. Agrociencia 47(6):567-568.

Li, H., H. Wang, L. Yu, M. Wang, S. Liu, L. Sun, and Q. Chen. 2015. Effects of supplementation of rumen-protected choline on growth performance, meat quality and gene expression in longissimus dorsi muscle of lambs. Archives of Animal Nutrition 69(5): 340-350. https://doi.org/10.1080/1745039X.2015.1073001

Liu, Y.Z., X. Chen, W. Zhao, M. Lang, X. F. Zhang, T. Wang, Y.G. Zhen, and G. X. Qin. 2019. Effects of yeast culture supplementation and the ratio of non-structural carbohydrate to fat on rumen fermentation parameters and bacterial-community composition in sheep. Animal Feed Science and Technology 249:62-75. https://doi.org/10.1016/j.anifeedsci.2019.02.003

Martínez-Aispuro, J. A., G.D. Mendoza, J.L. Cordero-Mora, M.A. Ayala-Monter, M.T. Sánchez-Torres, J.L. Figueroa-Velasco, G. Vázquez-Silva, and A. Gloria-Trujillo. 2019. Evaluation of an herbal choline feed plant additive in lamb feedlot rations. Revista Brasileira De Zootecnia 48: e20190020. https://doi.org/10.1590/rbz4820190020

Martínez, G.J.A., L.J.C. García, G.P.A. Hernández, M.G.D. Mendoza, V. A. Vázquez, M.M. Delgadillo, and R.H.A. Lee. 2021. Changes in productive performance, blood metabolites and hematological parameters of growing lambs supplemented with two sources of choline. Indian Journal of Animal Research 1-6. https://arccjournals.com/journal/indian-journal-of-animal-research/B-1385

Mendoza, G.D., M. F. Oviedo, J.M. Pinos, H. A. Lee, A. Vázquez, R. Flores, F. Pérez, A. Roque, and O. Cifuentes. 2019. Milk production in dairy cows supplemented with herbal choline and methionine. Revista de la Facultad de Ciencias Agrarias 52(1): 332-343. https://revistas.uncu.edu.ar/ojs/index.php/RFCA/article/view/3076

NRC. 2007. National Reseach Council. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Washington, DC: The National Academies Press.

Okhuarobo, A., J. E. Falodun, O. Erharuyi, V. Imieje, A. Falodun, and P. Langer. 2014. Harnessing the medicinal properties of Andrographis paniculata for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pacific Journal of Tropical Disease 4(3): 213-222. https://doi.org/10.1016/S2222-1808(14)60509-0

Rodríguez, V., A. C. Lizarazo, S. Ferraro, N. Suárez, L. A. Miranda, and D. G. Mendoza. 2018. Effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. South African Journal of Animal Science 48(3): 427-434. http://dx.doi.org/10.4314/sajas.v48i3.3

SAS. 2011. Statistical Analysis System User's Guide. Cary, NC: USA. SAS institute Inc.

Shinde, A. K., S.K. Sankhyan, R. Meena, and R.K. Regar. 2013. Effect of feed supplementation with copper-and zinc salts on the growth, wool yield, nutrient utilization, blood constituents and mineral profile of Malpura lambs. Agricultural Science Research Journal 3(9): 284-291.

Suárez, S.N.E, Lee, R.H.A, C.A.C. Lizarazo, M.G.D. Mendoza, R.G. Espinosa, G. P. A. Hernández, L.J.C. García, G. J. A. Martínez, G. F. Álvarez, and J.J.A. Roque. 2023. Effect of the supplementation using an herbal mixture as a choline source during early gestation in Rambouillet Ewes. Animals 13(4):645. https://doi.org/10.3390/ani13040645

Supriyati, K., I.G. Budiarsana, L. Praharani, R. Krisnan, and I.K. Sutama. 2016. Effect of choline chloride supplementation on milk production and milk composition of Etawah grade goats. Journal Animal Science and Technology 58: 30. https://doi.org/10.1186/s40781-016-0113-5

Tatke, P.A., S. S. Desai, and S.Y. Gabhe. 2014. Marker Based Standardization of Extracts and Formulations of Achyranthes aspera. International Journal of Pharmacognos and Phytochemistry Research 6(2): 324-327. https://www.researchgate.net/publication/285513788_Marker_based_standardization_of_extracts_and_formulations_of_Achyranthes_aspera

Upadhaya, S.D., and I. H. Kim. 2017. Efficacy of phytogenic feed additive on performance, production, and health status of monogastric animals - A review. Annals of Animal Science 17(4):929–948. https://doi.org/10.1515/aoas-2016-0079

Van Soest, P.J., J. B. Robertson, and B.A. Lewis. 1991. Methods of dietary fiber, neutral detergent fiber and nonstharch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74(10): 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Zhou, Z., M. Vailati-Riboni, E. Trevisi, J. K. Drackley, D. N. Luchini, and J. J. Loor. 2006. Better postpartal performance in dairy cows supplemented with rumen protected methionine compared with choline during the peripartal period. Journal of Dairy Science 99(11): 1–17. http://dx.doi.org/10.3168/jds.2015-10525