








Evaluation of an herbal choline feed plant additive in lamb feedlot rations

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ABSTRACT - An experiment was carried out to evaluate the effect of an herbal choline feed plant additive on the productive parameters and blood metabolites of finishing lambs. Forty male Hampshire × Suffolk lambs (initial body weight of 30.36±3.75 kg) were assigned according to a completely randomized design. Treatments consisted of dietary inclusion of the herbal additive BioCholine at 0, 3, 6, and 9 g/kg dry matter for 56 days. A linear response for herbal choline dose was observed for daily gain, final body weight, feed conversion, blood glucose, and blood phosphatidylcholine as herbal choline level was increased in the diet. Total protein, globulins, and low-density lipoproteins showed a quadratic effect, and there were no effects on intake, *Longissimus dorsi* area, back fat, blood cholesterol, triglycerides, high-density lipoproteins, or albumins. The inclusion of the herbal choline feed plant additive improved daily gain and feed efficiency of finishing lambs. The choline feed plant additive is a source that can be used to meet choline requirements in small ruminants, as demonstrated by blood phosphatidylcholine and lamb performance.

Keywords: nutraceutical, phosphatidylcholine, phospholipid, phytobiotics



Introduction

Choline is a water-soluble vitamin of the B complex that is required by lambs, and its metabolites are important for protein synthesis, phospholipids, acetylcholine, and liver fat metabolism (NRC, 2007; Li and Vance, 2008). Dietary choline is degraded extensively in the rumen and very little escapes rumen degradation (Baldi and Pinotti, 2006), and even when sources of ruminally protected choline (RPC) are available, they are usually not included in lamb diets because a choline requirement has not been firmly established even though its dietary inclusion may improve productive performance (NRC, 2007).

Ruminally protected choline chloride has been the most frequently evaluated source of choline for small ruminants (Bryant et al., 1999; Godinez-Cruz et al., 2015; Tsiplakou et al., 2016; Habeeb et al., 2017), and doses above 2.5 g/kg DM, equivalent to an intake of over 3 g/d for lambs, showed adverse effects on lamb performance (Li et al., 2015). However, the causes of the impaired performance have not been identified.

One problem with RPC is that commercial products differ in their choline content and rumen degradability (Kung et al., 2003; Brusemeister and Sudekum, 2006). Furthermore, only up to 61% of

choline chloride reaching the duodenum is absorbed (De Veth et al., 2016). An alternative to replace RPC products is a feed plant additive with choline conjugates (BioCholine) that has resistance to ruminal degradation, as confirmed by results with growing lambs (Godinez-Cruz et al., 2015) and milk production of ewes (Crosby et al., 2017). However, inclusion levels of BioCholine may not be extrapolated from graded levels of RPC (Li et al., 2015), because the herbal product contains mainly phosphatidylcholine (PCho).

Therefore, the objective of this experiment was to establish the optimal level of supplemented choline of herbal origin in diets of finishing lambs based on productive performance and some blood metabolite indicators of energy metabolism.

Material and Methods

Research on animals was conducted according to the institutional committee on animal use. The experiment was carried out in Montecillo, State of Mexico, Mexico located at 98°54'11" W, 19°27'38" N, and 2250 m altitude. The mean annual temperature at this site is 15.9 °C.

Forty male Hampshire × Suffolk lambs, initial body weight (BW) = 30.36±3.75 kg, were assigned according to a completely randomized design to one of four treatments, which consisted of doses of choline feed plant additive of 0, 3, 6, and 9 g/kg DM (BioCholine Powder®, Technofeed Mexico, Querétaro, México) in a basal diet formulated for a daily gain of 300 g (Table 1; NRC, 2007). The herbal product contains 16 g/kg of total conjugates of choline and is a polyherbal mixture based on *Achyranthes aspera*, *Trachyspermum ammi*, *Azadirachta indica*, *Citrullus colocynthis*, and *Andrographis paniculata*.

Lambs were housed in individual metabolic crates equipped with a single feeder and nipple drinker. Before the experiment, lambs were dewormed (Closantil® 5%, 20 mg/kg BW orally) and vaccinated against *Clostridium chauvoei*, *Clostridium septicum*, *Clostridium novyi*, *Clostridium sordelli*, *Clostridium perfringens*, *Pasteurella multocida* type A, *Pasteurella multocida* type D, and *Pasteurella haemolytica* (Bobact® 8, 2.0 mL/lamb). Feed was offered at 08.00 and 15.00 h; water and feed were provided *ad libitum*. Lambs were previously fed a diet with alfalfa hay and grain in a 50:50 ratio; therefore, the

Table 1 - Ingredient and nutrient composition of experimental diets for finishing lambs

Item	Choline feed plant additive (g/kg DM)			
	0	3	6	9
Ingredient (g/kg)				
Corn grain	567.8	568.8	565.8	562.8
Soybean meal	227.3	223.3	223.3	223.3
Alfalfa hay	100.5	100.5	100.5	100.5
Oat straw	50.0	50.0	50.0	50.0
Cane molasses	40.0	40.0	40.0	40.0
Feed plant additive ¹	0.0	3.0	6.0	9.0
Calcium carbonate	2.4	2.4	2.4	2.4
Sodium chloride	2.0	2.0	2.0	2.0
Minerals and vitamins premix ²	1.00	1.00	1.00	1.00
Nutrient composition calculated ³				
Metabolizable energy (Mcal/kg)	2.90	2.90	2.88	2.86
Crude protein	185.5	185.6	184.8	186.9
Acid detergent fiber	141.5	142.6	147.0	145.4
Calcium	7.8	8.4	7.3	7.7
Phosphorus	5.4	4.9	5.3	5.2

¹ BioCholine Powder® (Nuproxa Mexico, Querétaro, México).

² Superbayphos® Bayer, composition per kg of premix: phosphorus, 100 g; calcium, 120 g; Iron, 5 g; magnesium, 1 g; copper, 1.5 g; zinc, 1.2 g; manganese, 0.55 g; cobalt, 0.5 g; iodine, 0.2 g; selenium, 0.2 mg; vitamin A, 50,000 UI.

³ Calculated using values for feed ingredients from the NRC (2007).

animals had a period of eight days to adapt to the experimental diets. The experimental phase lasted 56 days. Lambs were weighed in preprandial conditions on two consecutive days at the beginning (days 0 and 1) and end of the experiment (days 55 and 56). Samples of feed were analyzed for crude protein by macro Kjeldahl (AOAC, 2005); calcium and phosphorus by atomic absorption spectrophotometry with a Perkin Elmer 4000 Model (Series Lambda 2, Perkin Elmer Inc., Norwalk, CT, USA); and acid detergent fiber, using the Van Soest et al. (1991) procedures.

The recorded variables were daily feed intake, feed:gain ratio, initial and final BW, and average daily gain (ADG) calculated as (final BW – initial BW) / (days in experiment). Back fat thickness and *Longissimus dorsi* area were measured using a real time ultrasound Sonovet 600 (Medison, Inc., Cypress, California, USA) with a 7.5 Mhz transducer between the 12th and 13th ribs on day 55 of the experiment (Silva et al., 2005). On day 56, blood samples (5 mL; preprandial at 08.00 h) were collected from the jugular vein by puncture, using vacutainer tubes without anticoagulant (BD Vacutainer), placed immediately under refrigeration (4 °C), and then centrifuged (Sigma 2-16 k, Germany) at 3500 × g for 20 min to obtain blood serum; this was stored in Eppendorf tubes and kept in a freezer (Sanyo MDF-436, USA) at –20 °C until analysis of total cholesterol, glucose, total protein, albumin, high- (HDL) and low-density (LDL) lipoprotein, and PCho (Takayama et al., 1977) using Spinreact kits (Barcelona, Spain).

Data were analyzed using the GLM procedure of SAS (Statistical Analysis System, version 9.1), and linear and quadratic effects were tested to evaluate the effects of choline feed plant additive level. Each lamb was considered an experimental unit. The initial BW was used as a covariate for final BW, ADG, and DM intake. The models used were:

$$Y_{ij} = \mu + \tau_i + e_{ij}$$

$$Y_{ij} = \beta_0 + \beta_1 x_{ij} + \tau_i + e_{ij},$$

in which μ is the mean value, τ_i is the fixed treatment effect, Y_{ij} is the observation j in the treatment i , β_0 is the intercept, β_1 the regression coefficient, x_{ij} is the covariate with mean μ_x , and e_{ij} is the error term. Response variables were also tested for simple correlations.

Results

Final BW, ADG, and feed conversion were improved linearly ($P < 0.10$; Table 2) by the inclusion of choline feed plant additive, whereas intake, fat thickness, and *Longissimus dorsi* area were not affected by herbal choline levels ($P > 0.05$). A linear response was observed ($P < 0.10$) for blood glucose ($P < 0.01$) and blood PCho ($P < 0.10$) as herbal choline was increased in the diet. Blood glucose was negatively associated with daily gain ($r = 0-0.52$, $P < 0.0004$), but other metabolites were not correlated with lamb performance. Total protein ($P < 0.01$), globulins ($P < 0.04$), and LDL ($P < 0.10$) showed a quadratic response ($P < 0.10$), whereas no effect was detected ($P > 0.05$) on blood cholesterol, triglycerides, HDL, or albumins (Table 3).

Table 2 - Least square means of lamb performance traits by level of herbal choline

Item	Choline feed plant additive (g/kg DM)				SEM	P-value	
	0	3	6	9		Linear	Quadratic
Initial BW (kg)	30.57	30.19	30.24	30.44	1.23	0.84	0.88
Final BW (kg)	52.37	52.12	54.28	54.16	1.03	0.10	0.95
ADG (kg/d)	0.392	0.389	0.427	0.426	0.018	0.10	0.97
DMI (kg/d)	1.73	1.67	1.79	1.64	0.06	0.60	0.51
Feed conversion	4.43	4.36	4.24	3.92	0.12	0.004	0.34
Backfat (mm)	4.19	4.00	4.10	4.40	0.20	0.43	0.24
Chop area (mm ²)	1052	1186	1156	1157	66	0.34	0.31

BW - body weight; ADG - average daily gain; DMI - dry matter intake; SEM - standard error of the mean.

Table 3 - Least square means of lamb metabolites means by effect of herbal choline

Item	Choline feed plant additive (g/kg DM)				SEM	P-value	
	0	3	6	9		Linear	Quadratic
Cholesterol (mg/dL)	160.15	187.36	168.11	171.04	11.13	0.78	0.28
PCho (mg/dL)	103.44	108.40	126.11	112.31	6.11	0.10	0.13
Triglycerides (mg/dL)	72.71	81.93	78.19	73.84	5.97	0.98	0.26
HDL (mg/dL)	46.40	44.65	44.98	47.46	3.31	0.81	0.52
LDL (mg/dL)	27.75	41.16	44.08	34.73	6.98	0.44	0.10
Glucose (mg/dL)	87.57	91.96	117.62	132.13	3.05	0.001	0.10
Total protein (g/dL)	6.59	6.15	5.94	6.81	0.38	0.79	0.01
Albumins (g/dL)	2.64	3.09	2.56	2.80	0.12	0.88	0.38
Globulins (g/dL)	3.94	3.05	3.37	4.00	0.35	0.74	0.04
Albumins/Globulins	0.77	1.13	0.78	0.88	0.14	0.98	0.36

PCho - phosphatidylcholine; HDL - high-density lipoproteins; LDL - low-density lipoproteins; SEM - standard error of the mean.

Discussion

Li et al. (2015) showed an erratic response of intake but a quadratic response of gain, in which the maximum gain and the best feed conversion were observed with 2.5 g/kg of RPC, unlike in the current experiment, in which gain and feed conversion showed no quadratic response with the best efficiency at 9 g/kg of BioCholine. Bryant et al. (1999) observed that ADG improved with 2.5 g/kg DM RPC in the diet of lambs, but there was no response at higher levels (5 and 10 g/kg DM). The addition of choline chloride in RPC at lower dietary concentrations in beef cattle and goats improved feed conversion and ADG (Pinotti et al., 2009; Habeeb et al., 2017). However, when choline was combined with RPC the productive performance was impaired (El-Gendy et al., 2012) or showed no response in daily gain (Rodríguez-Guerrero et al., 2018). The active mechanism by which excess choline chloride negatively affects animal performance is unclear; one possibility is that it surpasses the ability to metabolize choline at the cellular level, resulting in the accumulation of phosphocholine, as observed in breast cancer cells (Eliyahu et al., 2007).

Previous evaluations of the same herbal choline feed plant additive showed that 1 g of the herbal choline product resulted in the same productive response as in lambs that received 4 g of RPC (Godinez-Cruz et al., 2015; Crosby et al., 2017), possibly because the herbal product contains phospholipids (mainly PCho) rather than choline chloride. The metabolic pathway of PCho in the body differs from that of free choline, as PCho requires less energy expenditure and does not require several metabolic processes to become available to the cells. Free choline requires transporters to enter into the cells (some require ATP), then requires a molecule of ATP in the formation of phosphocholine, followed by a “rate-limiting” step in the conversion of phosphocholine to CDP (cytidine diphosphocholine)-choline, which determines the biosynthetic flux from choline to PCho (Fagone et al., 2013). In contrast, when PCho is absorbed with other products of fat digestion, it is transported in the blood as lipoproteins and is available for cells and tissues (Tocher et al., 2008).

In previous experiments with lambs, carcass variables related to fat deposition did not change in response to levels of RPC (Bryant et al., 1999; Li et al., 2015) despite the lipotropic effect of choline (Piepenbrink and Overton, 2003) and the fact that the oxidized form of choline (betaine) has consistently been shown to reduce body fat deposition in other species (Eklund et al., 2005).

Studies on lambs confirmed that the inclusion of RPC or the herbal choline in lamb diets increased NEFA (non-esterified fatty acid) concentrations in plasma (Bryant et al., 1999; Rodríguez-Guerrero et al., 2018). Changes in serum cholesterol were reported in one experiment in which the diet had proportionally more forage (Rodríguez-Guerrero et al., 2018) than in experiments in which no differences were detected (Bryant et al., 1999; Li et al., 2015).

The changes in PCho in blood confirm the protected nature of the herbal choline previously suggested by productive responses (Godinez-Cruz et al., 2015; Crosby et al., 2017). In humans, plasma choline

levels were reduced by dietary choline restriction (Zeisel et al., 1991). Meanwhile, experiments on rats showed that dietary PCho was more effective at increasing blood PCho in suckled rat offspring than choline bitartrate (Lewis et al., 2016).

In growing lambs, increments in blood glucose in response to herbal choline were observed by Rodríguez-Guerrero et al. (2018), and to RPC in growing goats by Habeeb et al. (2017); liver glycogen was also increased in dairy cows fed RPC (Piepenbrink and Overton, 2003). Choline may act by altering the intracellular signaling of energy metabolism, as shown in studies on insulin-resistant mice, in which choline supplementation reduced glucose utilization for fatty acid and triglyceride synthesis and increased muscle glycogen (Taylor et al., 2017). Choline is associated with peroxisome proliferator-activated receptors (PPAR), which regulate adipogenesis and lipogenesis (Yu et al., 2003), as well as adiponectin, which plays an important role in the regulation of fatty acid and glucose metabolism.

Similar to the results of the current study, Li et al. (2015) reported an increased (linear and quadratic) response to RPC in lamb blood LDL, but they also detected a quadratic reduction in HDL. Cole et al. (2012) reported that impaired hepatic PCho biosynthesis reduces the levels of circulating VLDL (very low-density lipoproteins) and HDL through a reduction in the hepatic secretion of VLDL and by a low synthesis of VLDL, which influences the biological activity and gene expression that regulate lipoprotein production. However, changes in response to normal or higher intakes of choline have not been studied. Li et al. (2015) studied the effect of RPC on enzymes involved in lipogenesis, but the collection of data on genes that regulate hepatic lipoproteins in ruminants requires further research.

Differing levels of choline had no effect on blood triglycerides as observed by Li et al. (2015), and Rodríguez-Guerrero et al. (2018) also reported no response to herbal choline, except when it was combined with ruminally protected methionine, which increased triglyceride levels. The role of choline in the liver via PCho as a metabolite necessary for the packaging and export of triglycerides in VLDL has been recognized (Noga and Vance, 2003).

The albumin:globulin ratio did not indicate any abnormality or immune alteration. Lambs fed ration supplemented with herbal choline showed no change in serum albumin and total protein concentration (Rodríguez-Guerrero et al., 2018). However, growing goats under heat stress exhibited increased total protein and globulin, phospholipids, and plasma choline and improved daily gain (Habeeb et al., 2017). Studies have shown that PCho and choline can stimulate the immune response (Lewis et al., 2016).

The quadratic trend in blood PCho and ADG values suggest that the optimal supplemental level of feed additive is around 6 g/kg DM; the maximum blood PCho was estimated to occur at 5.91 g/kg DM, as derived from the quadratic equation. The plants that make up the feed plant additive contain other metabolites that could affect ruminal fermentation, possibly explaining the lack of response at 9 g/kg; Mendoza et al. (2019) reported that the feed additive contains trans-2-Undecenal, 8-p-menthane diamine, 4-vinylguaicol, β -pinene, and p-cresol, which have bacteriostatic and bactericidal effects (Esatbeyoglu et al., 2015; Widhalm et al., 2016). Higher dietary levels of choline feed plant additive require further evaluation.

Conclusions

Choline feed plant additive can be included at 6 g/kg to improve lamb performance in finishing diets. The blood changes in phosphatidylcholine confirm that the herbal product contains choline conjugates resistant to rumen degradation. Herbal choline has important hypoglycemic effects. The results highlight the importance of this alternative source of choline for use in ruminant diets to meet choline requirements, as demonstrated by blood phosphatidylcholine, lamb growth, and feed efficiency.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: J.A. Martínez-Aispuro and G.D. Mendoza. Data curation: J.L. Cordero-Mora and M.A. Ayala-Monter. Formal analysis: J.A. Martínez-Aispuro, G. Vázquez-Silva and A. Gloria-Trujillo. Funding acquisition: G.D. Mendoza. Investigation: J.A. Martínez-Aispuro and G.D. Mendoza. Methodology: J.A. Martínez-Aispuro, G.D. Mendoza and A. Gloria-Trujillo. Project administration: G.D. Mendoza and J.L. Cordero-Mora. Resources: G.D. Mendoza, M.T. Sánchez-Torres and J.L. Figueroa-Velasco. Software: J.A. Martínez-Aispuro, M.A. Ayala-Monter and A. Gloria-Trujillo. Supervision: J.A. Martínez-Aispuro and G.D. Mendoza. Validation: G.D. Mendoza and A. Gloria-Trujillo. Visualization: J.A. Martínez-Aispuro and G.D. Mendoza. Writing-original draft: J.A. Martínez-Aispuro, G.D. Mendoza and M.A. Ayala-Monter. Writing-review & editing: J.A. Martínez-Aispuro and G.D. Mendoza.

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