Evaluation of two sources of choline on reproductive variables in primiparous ewes

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ABSTRACT

Objective: to evaluate the reproductive response of primiparous ewes supplemented with herbal choline and synthetic choline during an estrus synchronization protocol.

Design/Methodology/Approach: ninety-two 15-month-old primiparous ewes were used, with initial body weight of 50±1.25 kg. The ewes were distributed randomly in three (T) treatments: T1 (basic diet without choline), T2 (basic diet + 4 g of herbal choline), and T3 (basic diet + 4 g of synthetic choline).

Results: there were no differences among treatments for presentation and onset of estrus, the pregnancy rate, prolificacy, type of delivery (lambing), and insulin concentration ($p>0.05$). But the concentration of progesterone did decrease ($p\leq0.05$) before estrus with the addition of herbal choline to the diet.

Limitations/Implications of the study: the dietary addition of herbal or synthetic choline (4 g d^{-1}) did not improve reproductive variables, because the level of supplementation was low. Or else, the animals synthesized or consumed a sufficient amount of choline for their metabolic functions.

Findings/Conclusions: the inclusion of herbal or synthetic choline during synchronized estrus in primiparous ewes did not alter the reproductive variables, while progesterone concentrations were modified due to herbal choline supplementation.

Keywords: herbal, synthetic, progesterone, reproduction.

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INTRODUCTION

Choline is considered a conditionally essential vitamin in sheep, which is involved in the synthesis of proteins, phospholipids, acetylcholine, bone growth, membrane integrity, signaling functions, and development of the nervous system of the fetus. It is also an essential factor in the metabolism of fat in the liver and the regulation of methylation processes (NRC, 2007).

Because dietary choline is extensively degraded in the rumen and a limited amount passes through to the gut (Jayaprakash *et al.*, 2016), supplementation with choline protected from rumen degradation reduces deficiency, and improves productive behavior, health, and reproductive aspects in sheep (Li *et al.*, 2015; Tsiplakou *et al.*, 2016; Rodríguez-Guerrero *et al.*, 2018; Suárez-Suárez *et al.*, 2023).

Synthetic (CS) and herbal (CH) choline sources are available on the market; however, they are not regularly included in sheep feed. Choline chloride is the synthetic source commonly used in animal diets; however, under poor storage conditions, high hygroscopicity can accelerate oxidation of food vitamins and premixes (Tavcar-Kalcher and Vengust, 2007), low bioavailability (40-80%), and toxicity of secondary metabolic products (Jayaprakash *et al.*, 2016).

In addition, recent animal production restricts the use of synthetic compounds, which is why it is sought to incorporate alternative natural dietary supplements (from plants) into ruminant diets that are relatively non-toxic and environmentally friendly. Therefore, CS can be replaced by food additives from plants containing phosphatidylcholine, which show natural resistance to rumen degradation (Gutiérrez *et al.*, 2019). The objective of this research was to compare the effect of the addition of herbal or synthetic choline on the reproductive variables of primiparous ewes during an estrus synchronization protocol.

MATERIALS AND METHODS

The research was established at the Experimental Farm of the Colegio de Postgraduados, Texcoco, State of Mexico (19° 27' 18" N and 98°5 4' 26" W), at an altitude of 2220 m; the climate is temperate subhumid with rainfall in summer. The sheep were managed in accordance with the Official Mexican Standard NOM-062-ZOO-1999 for the use and care of animals intended for research.

Ninety-two 15-month-old primiparous sheep (Dorset×Kathadin), with an average initial live weight of 50±1.25 kg and a body condition of 3 on a scale of 1 to 5 were used in the experiment. Zootechnical management before the start of the experiment consisted of deworming, vitaminizing (A, D and E), bacterin application (BOBACT® 8 ways), shearing and hoof-trimming.

The ewes were subjected to an estrus synchronization protocol (Figure 1) by applying synthetic hormones to submit them to a reproductive program during the months of October to December (reproductive season). The ewes were fed a base diet consisting of oat hay and dehydrated alfalfa on free-access, supplemented with 250 g of concentrate per animal per day; which was added with choline during the synchronization and mating period (36 days: 15 days before mating, and 21 days after mating).

The treatments (T) consisted of supplementing different types and dietary concentrations of choline incorporated into the concentrate. Base diet (forage+concentrate) without the addition of choline (T1, n=30); Base diet+4 g of herbal choline (T2; n=31); Base diet+4 g synthetic choline (T3; n=31). The source of herbal choline was the commercial product BioCholine® (Indian Herbs and Technofeed, Mexico) and the synthetic source was 50% choline chloride (Orffa Elovitals, Belgium). The composition of the concentrate (g kg^{-1}

DM) was as follows: corn (806.6), soybean paste (131.5), common salt (2), molasses (50), and mineral premix (10).

Regarding reproductive management (Figure 1), estrus presynchronization was performed by two applications of 125 μg of prostaglandin F2α (cloprostenol®), on days −8 and 0 of the protocol. Six days after the second application of PGF2α, an intravaginal sponge impregnated with 20 mg of progesterone (sponges with crolonone acetate) was inserted for 11 days.

The females received three mating events; the first at the beginning of the estrus, and two subsequent at intervals of 12 h. Return to estrus was detected in two periods (morning and afternoon) between days 14 and 17 after synchronized estrus and mating. The diagnosis of pregnancy was made 31 days after the last mating event (SONOVET 600® ultrasound with 7.5 Mhz transrectal linear transducer). The evaluation timeline is shown from the start of the estrus synchronization protocol (Figure 1).

Blood samples (5 mL) were collected from the jugular vein prior to placement of the sponge, and thereafter every 48 h, to determine the concentration of progesterone during the estrus synchronization period. Insulin concentrations (INS) were monitored in fasting conditions, on days 1 and 36 of the choline addition period. All samples were centrifuged for 20 min at 2500 x*g* to separate the blood serum, which was stored at −20 °C until hormonal analysis. To determine P_4 concentrations, a radioimmunoassay (RIA) was performed with a commercial kit PROGEST-CTRIA® (CIS-BIO INTERNATIONAL, France) with intra- and inter-assay coefficients of variation (CV) of 4.1 and 8.7, respectively, and sensitivity of 0.05 ng mL^{-1}. Plasma insulin analysis was also performed by radioimmunoassay (RIA) with a sensitivity of 4.09 ng mL^{-1} and intra- and inter-assay coefficients of variation of 3.2 and 4.6%.

The experimental design was completely randomized, each ewe was considered as an experimental unit. The presentation of estrus, percentage of gestation, prolificacy index and type of delivery (lambing) were analyzed with an X^2 test with the PROC FREQ procedure. The analysis of variance of estrus onset and insulin concentration was executed

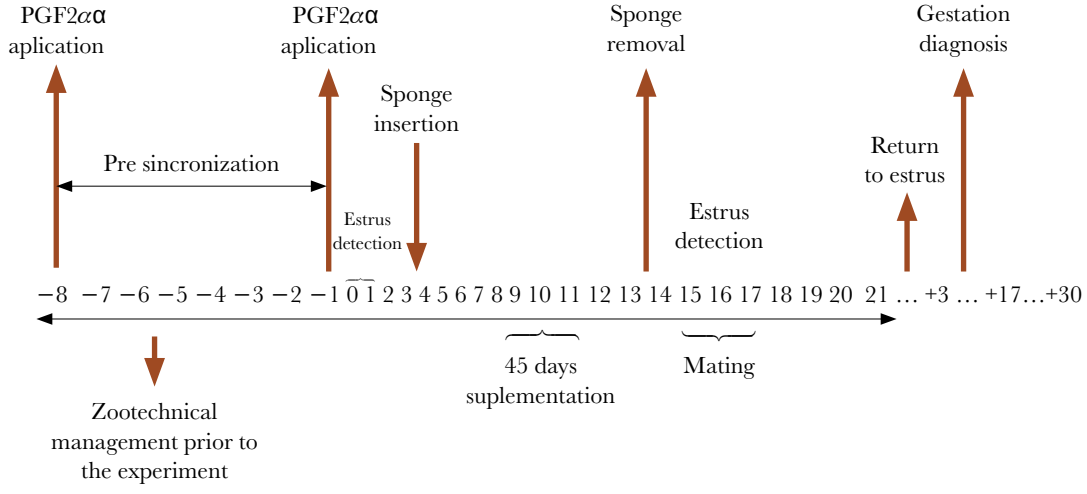

Figure 1. Experimental protocol in female sheep fed with herbal choline and synthetic choline.

with the PROC GLM procedure ($p \leq 0.05$). For the concentration of progesterone, the analysis of variance of repeated measurements over time was performed using the PROC MIXED procedure, which included treatment and day as fixed effects, as well as their interaction. For this procedure, the covariance structure was modeled with the effect of the ewes within the group. The comparison of means was performed with Tukey's test ($p \leq 0.05$). All variables were analyzed in SAS® 5.0 for Windows (SAS Institute, Inc., NC, USA).

RESULTS AND DISCUSSION

The presentation of estrus, estrus onset, and percentage of gestation (Table 1) were not influenced ($p > 0.05$) by the dietary addition of herbal or synthetic choline. Ardalan *et al.* (2009) noted that supplementing 60 g d^{-1} choline in cows in the first third of lactation period does not improve the percentage of pregnant cows. However, in Zaraibi goats, there is evidence that supplementation with CS (10, 20 and 40 g d^{-1}) increased the pregnancy rate (90-100%) compared to the control group (80%) (Habeeb *et al.*, 2018).

Choline supplementation to cows in the transition stage (before and after calving) positively affects the appearance of first post-delivery heat, open days, the number of services, and the pregnancy rate (Acharya *et al.*, 2019; Gutiérrez *et al.*, 2019; Mecionyte *et al.*, 2022). During the transition stage (dry period-early lactation), dairy cows often experience a negative energy balance, leading to mobilization of energy reserves, decreased dry matter intake, higher levels of non-esterified fatty acids (NEFAs), and consequent lower milk production. This predisposes early lactating cows to metabolic diseases, such as fatty liver syndrome and ketosis; in addition to reproductive problems.

Rumen-protected choline supplementation is a strategy to restrict the negative effects associated with negative energy balance in early lactating cows. What results in increased follicular development and fertility, as choline deficiency causes a decrease in the production of FSH and LH hormones. However, in research conducted where herbal or synthetic choline are evaluated, there seems to be no consistency in the benefits to the reproductive response (Humer *et al.*, 2019).

In the study conducted by Acosta *et al.* (2016) although CS supplementation to cows during the transition period did not affect the days to first ovulation; choline supplementation reduced the expression of mRNA factors (TNF, TLR4 and IL1-β) in follicular cells, which are associated with increased infertility. The difference in the response, this is the improvement of reproductive variables, between those dairy cows in the transition stage, and breeding sheep may be due to the fact that the sheep used our study presented a good body condition throughout the evaluation.

Dietary choline supplementation to dairy cows had shown lower incidence of metritis, endometritis, pyometra, placental retention (Furken & Hoedemaker, 2014), and reduced the presence of metritis-associated pathogens (Marques *et al.*, 2023).

Prolificacy. The addition of herbal and synthetic choline did not influence ($p > 0.05$) the prolificacy index and the type of lambing (single and double), which contrasts with what was found by Habeeb *et al.* (2018) who observed that adding CS (20 and 40 g d^{-1}) to the diet of Zaraibi goats increased the number of double and triple kiddings. Also, studies

in cows indicated that the group added with CS had a higher incidence of twin births compared to the control group (Guretzky *et al.*, 2006).

Insulin concentrations. The addition of choline (herbal and synthetic) during estrus synchronization and days after mating did not modify the concentration ($p>0.05$) of insulin in sheep blood serum (Table 1). Because choline supplementation to fattening lambs increased glucose concentration (Crosby *et al.*, 2017), it was inferred that there could potentially be an increase in insulin, however, this did not happen. In another study, dietary supplementation of CS (18.8% choline) with 50 or 100 g d^{-1} per animal in multiparous Holstein cows in the transition stage resulted in a higher insulin concentration compared to the control treatment (Leiva *et al.*, 2015).

Progesterone (P_4; Figure 2). The P_4 levels of sheep that were supplemented with herbal choline decreased ($p\leq0.05$) during the days that the exogenous source of P_4 (intravaginal sponge) was available compared to the control treatment. While ewes supplemented with synthetic choline only tended to reduce the level of P_4, without detecting differences ($p>0.05$) compared to the control treatment or the treatment with herbal choline; except on day 2 before the removal of the intravaginal sponge, where a clear decrease in the concentration of P_4 was observed compared to the control treatment ($p\leq0.05$). Generally speaking, it can be seen that choline supplementation (herbal or synthetic) tended to reduce the level of P_4 during the time that one external source of P_4 (sponge) was present in the vagina of the ewe.

Results obtained in this research differ from those expected, as there is evidence that CS supplementation in goats increased the concentration of P_4 (Habeeb *et al.*, 2018). Also, in peripartum cows (before and after calving) the first peak of P_4 was increased during days 21-24 postpartum (Mecionyte *et al.*, 2022). Although, in the study conducted by Acosta *et al.* (2016), CS supplementation to cows during the transition period did not affect estradiol and progesterone concentrations.

Since cholesterol is a precursor for P_4 synthesis, and there is evidence that CH supplementation (4 g d^{-1}) to fattening lambs increased cholesterol concentrations (Crosby *et al.*, 2017; Rodríguez-Guerrero *et al.*, 2018; Martínez-Aispuro *et al.*, 2022), we hypothesized that a higher cholesterol concentration would influence P_4 concentration. In spite that,

Table 1. Reproductive response of ewes supplemented with different choline sources.

Reproductive variables	Treatments		
	T1 (n=30)	**T2 (n=31)**	**T3 (n=31)**
Estrus onset (%)	93.33 (28/30)	93.55 (29/31)	96.77 (30/31)
Beginning of estrus (h)	43.28±2.57	42.00±2.48	42.07±2.57
Gestation (%)	93.33(28/30)	93.55 (29/31)	87.10 (27/31)
Prolificacy index	1.22 (33/27)	1.24 (36/29)	1.30 (34/26)
Single birth %	73.33 (22/30)	70.97 (22/31)	61.29 (19/31)
Twin birth %	16.67 (5/30)	22.58 (7/31)	19.35 (6/31)
Insulin (ng mL^{-1})	0.64±0.08	0.55±0.08	0.58±0.08

T1 (control): base diet + 0 g per ewe; T2 (herbal choline): base diet + 4 g choline per ewe; T3 (synthetic choline chloride): base diet + 4 g choline per ewe.

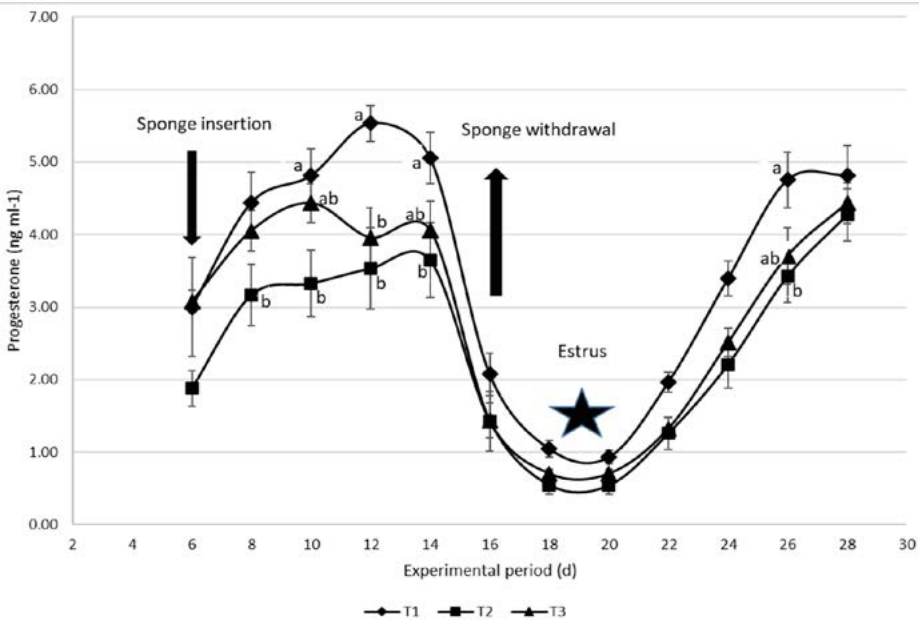

Figure 2. Concentration of progesterone (mean ± standard error) in choline-supplemented ewes. T1 (choline-free base diet), T2 (base diet + 4 g of herbal choline) and (T3) base diet + 4 g of synthetic choline. a,b values with different literal are statistically different ($p \leq 0.05$).

regarding peripartum dairy cows, there are inconsistencies in the reports, such as choline supplementation can either increase (Soltan *et al.*, 2012) or decrease (Sun *et al.*, 2016) cholesterol concentration.

CONCLUSIONS

The inclusion of herbal or synthetic choline in the diet during synchronized estrus of primiparous sheep did not alter reproductive variables. Meanwhile, progesterone concentrations were modified due to herbal choline supplementation. It is possible that the beneficial effect of dietary choline addition on reproductive variables would be manifested in sheep that were in a negative energy balance.

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REFERENCES

Acosta, D.A.V., Denicol, A.C., Tribulo, P., Rivelli, M.I., Skenandore, C., Zhou, Z., Luchini D., Correa M.N., & Cardoso, F.C. (2016). Effects of rumen-protected methionine and choline supplementation on the preimplantation embryo in Holstein cows. *Theriogenology 85*(9): 1669-1679. doi:10.1016/j.theriogenology.2016.01.024

Acharya, P., Lathwal, S.S., Baithalu, R., Patnaik, N., Thul, M.R., & Moharana, B. (2019). Supplementing rumen protected choline with green tea extract improves reproductive performances in transition karan Fries cows. *Indian J Anim Res* 54: 452-455. doi: 10.18805/ijar.B-3797

Ardalan, M., Rezayazdi, K., & Dehghan-Banadaky, M. (2009). Investigation on the effect of supplementing rumen-protected forms of methionine and choline on health situation and reproductive performance of Holstein dairy cows. *Pak J Biol Sci 12*(1): 69-73. doi: 10.3923/pjbs.2009.69.73

Crosby, M., Mendoza-Martínez, A., Relling, A., Vázquez, A., Lee-Rangel, H.A., Martinez, J.A., & Oviedo, M. (2017). Influence of supplemental choline on milk yield, fatty acid profile, and postpartum weight changes in suckling ewes. *J Dairy Sci* 100: 1265-1270. doi: 10.14202/vetworld.2024.1265-1270

Guretzky, N. J., Carlson, D. B., Garrett, J. E., & Drackley, J. K. (2006). Lipid metabolite profiles and milk production for Holstein and Jersey cows fed rumen-protected choline during the periparturient period. *J Dairy Sci 89*(1): 188-200. doi: 10.3168/jds.S0022-0302(06)72083-5

Gutiérrez, A., Sánchez, C., & Mendoza, G.D. (2019). Effect of including herbal choline in the diet of a dairy herd, a multiyear evaluation. *Emir J Food Agric* 31: 477-481. doi: 10.9755/ejfa.2019.v31.i6.1971

Furken, C., & Hoedemaker, M. (2014). Influence of feeding rumen-protected choline to transition dairy cows. Part 1: Metabolism and milk yield. *Tierarztl Praxis Ausg G Grosstiere/Nutztiere* 42: 11-21. doi: 10.1055/s-0038-1623206

Habeeb, A.A., Gad, A.E., Atta, M.A., & Mustafa, M.M. (2018). Effect of adding different levels of rumen protected choline to the diet on productive and reproductive performance of female goats and growth of their kids from birthing to weaning. *Anim Sci J 89*(2): 348-358. doi: 10.1111/asj.12932

Humer, E., Bruggeman, G., & Zebeli, Q. (2019). A meta-analysis on the impact of the supplementation of rumen-protected choline on the metabolic health and performance of dairy cattle. *Animals 9*(8): 566. doi: 10.3390/ani9080566

Jayaprakash, G., Sathiyabarathi, M., Robert, M.A., & Tamilmani, T. (2016). Rumen-protected choline: A significance effect on dairy cattle nutrition. Vet World 9:837-841. doi: 10.14202/vetworld.2016.837-841

Leiva, T., Cooke, R.F., Brandao, A.P., Marques, R.S., & Vasconcelos, J.L.M. (2015). Effects of rumen-protected choline supplementation on metabolic and performance responses of transition dairy cows. *J Anim Sci 93*(4): 1896-1904. doi: 10.2527/jas.2014-8606

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

Marques, T.C., Monteiro, H.F., Melo, D.B., Coelho Jr, W.M., Salman, S., Marques, L.R., Leao, K.M., Machado, V.S., Menta, P., Lima, F.S., Dubey, D., & Sun, F. (2023). Effect of rumen-protected choline on dairy cows' metabolism, immunity, lactation performance, and vaginal discharge microbiome. *J Dairy Sci 107*(5): 2864-2882 doi: 10.3168/jds.2023-23850

Martínez-Aispuro, J.A., Ayala M. M.A., Mendoza, M.G., Sánchez T. M.T., Cordero M. J.L., Figueroa V. J.L., & Martínez, C.I. (2022). Evaluation of herbal choline in productive performance and blood metabolites of ewes. *Agroproductividad 15*(9): 109-116. doi: 10.32854/agrop.v15i9.2261

Mecionyte, I., Palubinskas, G., Anskienė, L., Japertienė, R., Juodžentytė, R., & Žilaitis, V. (2022). The effect of supplementation of rumen-protected choline on reproductive and productive performances of dairy cows. *Animals 12*(14): 1807. doi: 10.3390/ani12141807

NRC (National Research Council). (2007). Nutrient requirements of small ruminants. Sheep, goats, cervids and world camelids. 7th Ed. National Academy Press. Washington DC, USA. 384p.

Rodríguez-Guerrero, V., Lizarazo, A. C., Ferraro, S., Miranda, L. A., Mendoza, G. D., & Suárez, N. (2018). Effect of herbal choline and rumen-protected methionine on lamb performance and blood metabolites. *S Afr J Anim Sci 48*(3): 427-434. doi: 10.4314/sajas.v48i3.3

Soltan, M.A., Mujalli, A.M., Mandour, M.A., & Abeer, M.E.S. (2012). Effect of dietary rumen protected methionine and/or choline supplementation on rumen fermentation characteristics and productive performance of early lactating cows. *Pakistan J Nutr* 11:221-230. http://pjbs.org/pjnonline/fin1710.pdf

Suárez-Suárez, N.E., Lee-Rangel, H.A., Lizarazo-Chaparro, A.C., Mendoza-Martínez, G.D., Espinosa-Reyes, G., Hernández-García, P.A., & Roque-Jiménez, J.A. (2023). Effect of the supplementation using an herbal mixture as a choline source during early gestation in Rambouillet ewes. *Animals 13*(4): 645. doi: 10.3390/ani13040645

Sun, F., Cao, Y., Cai, C., Li, S., Yu, C., & Yao, J. (2016). Regulation of nutritional metabolism in transition dairy cows: energy homeostasis and health in response to post-ruminal choline and methionine. *PloS one 11*(8): e0160659. doi: 10.1371/journal.pone.0160659

Tavcar-Kalcher, G., & Vengust, A. (2007). Stability of vitamins in premixes. *Anim Feed Sci Technol* 132:148-154. doi: 10.1016/j.anifeedsci.2006.03.001

Tsiplakou, E., Mavrommatis, A., Kalogeropoulos, T., Chatzikonstantinou, M., Koutsouli, P, Sotirakoglou. K., Labrou, N., & Zervas, G. (2016). The effect of dietary supplementation with rumen-protected methionine alone or in combination with rumen-protected choline and betaine on sheep milk and antioxidant capacity. *J Anim Physiol Anim Nutr* 101:1004-1013. doi: 10.1111/jpn.12537