



## Evaluation of Herbal Choline and Methionine Supplementation on a Milk Production in Dairy Cows

FULENCIO FERRETIZ-RODRÍGUEZ<sup>1</sup>, JOSE ALEJANDRO ROQUE-JIMÉNEZ<sup>1</sup>, HÉCTOR A. LEE-RANGEL<sup>1\*</sup>, GREGORIO ALVAREZ-FUENTES<sup>1</sup>, JUAN CARLOS GARCIA-LOPEZ<sup>1</sup>, ROLANDO ROJO-RUBIO<sup>2</sup>

<sup>1</sup>Facultad de Agronomía y Veterinaria, Centro de Biociencias, Instituto de Investigaciones en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, SLP, México; <sup>2</sup>Centro Universitario UAEM-Temascaltepec, Universidad Autónoma del Estado de México.

**Abstract** | Polyherbal additives represent an alternative to enhance the productivity in dairy cattle. Some herbal mixtures with conjugates of choline and methionine have improved milk yield in dairy cattle. An experiment was conducted to evaluate two herbal formulas as sources of choline and methionine on dairy cows production and metabolites change. Thirty-two Holstein cows (Body Condition Score  $3.01 \pm 0.16$ ) fed a basal diet, were randomly assigned to one of four treatments: Control diet, Biocholine (BIO) supplementation (15 grams/cow/day), Optimethionine (OP) supplementation (9 grams/cow/day), and BIO x OP supplementation, with a duration of 60 days. Optimethionine treatments decrease milk production ( $P \leq 0.05$ ), but fat corrected production was higher ( $P \leq 0.05$ ) when cows were supplemented with BIO. The chemical composition of the milk did not show differences between treatments. Plasma urea and cholesterol were not different between treatments, glucose shown a decrease ( $P \leq 0.05$ ) with supplementation. Aspartate transferase activity decrease ( $P \leq 0.05$ ) with BIO supplementation. Results indicate that the herbal BIO and OP present some productive and metabolic changes.

**Keywords** | Dairy cows, Choline, Methionine, Herbal formulas, Phytogenic sources

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**\*Correspondence** | Héctor Aarón Lee-Rangel, Facultad de Agronomía y Veterinaria, Centro de Biociencias, Instituto de Investigaciones en Zonas Desérticas, Universidad Autónoma de San Luis Potosí, SLP, México, CP 78000; **Email:** hector.lee@uaslp.mx

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## INTRODUCTION

Feed plant additives and their secondary metabolites used as additives could be oriented to modify rumen fermentation to increase nutrient utilization mainly in adult ruminant productions, in anthelmintic effects, and as immunity stimulants as well as in other physiological effects (Sanchez et al., 2021). Feed plant additives may represent an alternative means to improve health and production in dairy cattle. Still, it is necessary to identify effective products, adequate dosage, and optimal conditions to obtain the benefits (Ortega-Alvarez et al., 2020). Some polyherbal mixtures with choline and herbal methionine conjugates have improved milk yield in dairy cattle (Mendoza et al., 2020).

The conventional supplementation of ruminal-protected nutrients (amino acids or choline) has demonstrated

that they can improve milk yield (Martínez-García et al., 2021). However, increasing milk yield may also increase metabolic problems and other production diseases and reduce fertility (Ortega-Alvarez et al., 2020). Mendoza et al. (2020) mentioned that the use of biocholine and optimethionine in dairy cows can improve milk production due to the phytochemical content of these additives. In the period of transition to lactation, the cows present a negative energy and protein balance due to increased metabolic demand from the mammary gland (Lara et al., 2006). Since methyl donors must synthesize critical compounds such as phosphatidylcholine and carnitine in tissues, a negative methyl donor balance may also be a fundamental challenge for the transition dairy cow (Mendoza et al., 2020). Thus, the possibility to increase the duodenal flow should be evaluated for improving milk production in dairy cows by choline and methionine. Therefore, the aim of this experiment was to determine the effect of BIO and OP on

dairy cows' body score condition (BSC), milk production and composition, and changes on urea, cholesterol, and AST plasmatic.

The animal procedures were reviewed and approved by the Committee for the Ethical Use of Animals in Experiments of the Universidad Autonoma de San Luis Potosi (UASLP), according to the regulations and standards that are required by the Mexican government for the use of animals for a number of diverse activities (NOM-062-ZOO-1995). The animals were vaccinated with Bobact 8® and dewormed twice a year. The Dairy Farm have different biosecurity practices such sanitation, feed management, facility maintenance, manure handling, and disposal of dead animals. All cows were allowed in pens to high fresh and the high producers; the specific pen was designed in pie-shape are equipment with automatic watering system, the cows were milking twice a day (at 5:00h am and 15:00h pm) in a pipeline automatic machine programmed for recorder the individual production by approximately ten minutes. The milking parlor was disinfected daily, and the milking machine was cleaned every day. A professional pest control company gives the service to extermination, fumigation, and rodents prevention.

The experiment was conducted at the experimental farm of the UASLP (22°11' N, 100°56' O, 1850 m above sea level) with a mean temperature of 17.5°C. The experimental period has a duration of 60 days, where 32 Holstein cows (eight cows for treatment; BSC  $3.01 \pm 0.16$ ) with five days after parturition were assigned randomly to one of four treatments: (1) Control diet; (2) Biocholine (BIO) supplementation (15 grams/cow/day; Nuproxa México); (3) Optimethionine (OP) supplementation (9 grams/cow/day; Nuproxa Mexico); and (4) BIO x OP supplementation. The basal diet contains (16.3% CP with 6% of DRP, and 2.08 Mcal/kg ME) oat hay, alfalfa hay, rolled corn, and concentrate (65% forage, 35% concentrate). The treatments were given individually in the milking parlor at 06:00 hours.

Milk production was recorded daily, and its composition (fat, protein, lactose, total solids, and non-fatty solids) was characterized every seven days using the samples taken during the early and afternoon milking. Previous to analysis the samples were mixed and homogenized in a water bath during one minute until the samples reaches 29°C. Later the samples were analyzed using the Lactoscan Ultrasonic milk analyzer (Milkotronic®, Bulgaria). The 4% fat corrected milk (FCM) of each cow was calculated as follows:  $FCM = [(0.4 \text{ kg milk}) + (0.15 \text{ kg milk fat } \%)]$  by the formula proposed by DeFraen et al. (2006). The BSC was assessed twice on days 1 and 56 using the scale of 1 to 5, in increments of 0.25 according to Edmonson et al.

(1998), at the time of enrollment. At final of the trial a 32 blood samples were taken, after were centrifuged to obtain blood serum to analyze cholesterol, glucose, and aspartate aminotransferase (AST) using a Biochemistry Analyzer (Kontrolab®) Shapiro-Wilk tests was used to test normal distribution and data were analyzed as a completely randomized design with the R software testing for interaction of BIO x OP. The lactation days was tested as a covariate. The model for this design is  $Y_{ij} = \mu + \epsilon_{ij}$ , were  $Y_{ij}$  = response variable;  $\mu$  = mean effect of treatment and;  $\epsilon_{ij}$  = error.

Milk production showed significant changes ( $P \leq 0.05$ , Table 1) between treatments, where the OP supplementation treatment is lower than the others. Some authors reported that 12 to 15 grams per day of RPC increase milk production by about 7 to 8% above unsupplemented groups (Lima et al., 2012), representing approximately two kg/d of milk, like that observed in this experiment. Even when some experiments have used doses of 25 to 60 g of different choline chloride-based RPC sources (Zhou et al., 2016) or higher doses (Banevičius et al., 2016; Pinotti et al., 2005) concluded that the best responses to improve milk production are obtained when providing 12-20 g/day of RPC which is similar to the dose used with the herbal product.

**Table 1:** Effect of supplementation of BIO and OP on dairy cows performance, chemical milk composition, plasmatic urea, cholesterol, glucose and aspartate transferase concentration during 60 days.

	Control	BIO	OP	BIOxOP	SEM
Milk yield, kg/d	30.1 <sup>b</sup>	29.75 <sup>ab</sup>	27.01 <sup>a</sup>	28.6 <sup>ab</sup>	0.67
4% FCM, kg/d	29.9 <sup>a</sup>	32.24 <sup>b</sup>	29.19 <sup>a</sup>	26.78 <sup>a</sup>	0.82
<b>Body score condition</b>					
Day 1	2.81	3.00	3.18	2.87	0.13
Day 56	3.10	3.00	3.18	3.00	0.08
<b>Chemical composition, g/kg</b>					
Fat	3.97	4.81	4.53	3.57	0.51
Protein	2.97	2.91	3.03	3.02	0.08
Lactose	4.51	4.39	4.6	4.57	0.32
Total solids	8.19	8.12	8.33	8.31	0.19
<b>Plasmatic metabolites</b>					
Urea	19.1	17.7	18.75	21.09	5.09
Cholesterol	227.59	221.06	217.79	196.73	13.65
Glucose	103.31 <sup>b</sup>	91.28 <sup>a</sup>	91.58 <sup>a</sup>	89.52 <sup>a</sup>	15.23
Aspartate transferase	876.9 <sup>b</sup>	844.69 <sup>a</sup>	875.36 <sup>b</sup>	852.45 <sup>ab</sup>	18.92

SEM: standard error of the mean. <sup>a,b,c</sup> Rows with different literal differ ( $P < 0.05$ ).

Regarding rumen-protected methionine, [Lara et al. \(2006\)](#) evaluated various doses of rumen-protected methionine (RPM), and milk production increased up to 14% above the control with 16 grams per day and then decreased. A similar response was observed in first-stage heifers with doses of 14 to 16 g/d ([Ayala et al., 2010](#)). In both studies, milk protein concentration was increased by RPM. [Zhou et al. \(2016\)](#) observed a 9% increase in milk production with an estimated dose of 14 g/d and higher milk protein content. [Mendoza et al. \(2020\)](#) supplemented dairy cows with herbal choline and methionine. They found that a combination of these products increases the milk production until 1.07 liters/cow/day, contrary to what was observed in the present experiment.

Fat-corrected milk production increased in the treatment of cows supplemented with BIO ( $P \leq 0.01$ ), which coincides with that reported by [Mendoza et al. \(2020\)](#), who used the same sources in combination and reported a significant increase. [Patton et al. \(2015\)](#) report increases in production corrected for the effect of protected choline or methionine. However, few studies are evaluating herbal compounds. The chemical composition of milk showed no differences between treatments ( $P \leq 0.05$ , [Table 1](#)) for OP and BIO supplementation. Considering that methionine has been identified as one of the two most limiting amino acids for lactating cows ([NRC, 2001](#)), reaching the protected methionine requirement improves milk protein synthesis ([Zhou et al., 2016](#)) not happen in the present investigation.

No statistically significant differences were observed between treatments for plasma urea levels. Plasmatic urea decrease is associated with a higher pregnancy rate and lower early embryonic losses before pregnancy recognition ([Rajala-Schultz, 2001](#)). When the intake of degradable protein is high, or the input of degradable carbohydrates is low, the level of ammonium in the rumen increases and exceeds the amount that bacteria can utilize; when there is excess ammonium, it passes to the liver through the blood, where it is transformed and eliminated, resulting in increased urea levels in the blood ([Arias and Nesti, 1999](#)). The [NRC \(2001\)](#) points out that a low ratio between methionine and lysine in the diet may have an impact on the decrease of urea concentration in dairy cattle, the Lis: Met ratio should be 3:1, so that the requirements of these two amino acids are covered mainly in diets based on corn silage, such as the diet of this study ([Duque-Quintero et al., 2017](#)). The supplementation with BIO or OP supplementation does not have a significant effect on plasma cholesterol levels in Holstein cows. Phosphatidylcholine is a phospholipid component in mitochondrial membranes; its status affects liver function and lipid levels ([Roque-Jimenez et al., 2020](#)); therefore, choline may be suggested to increase hepatic lipid transport in low-fat diets. [Hajilou et al. \(2014\)](#), with a low-fat diet (2.66% fat), reported a reduction in

triglycerides with supplementation choline in Holstein steers, but cholesterol levels were not affected. The plasmatic concentration of glucose decrease ( $P \leq 0.05$ ) by the effect of BIO and OP. The hepatoprotective effects of choline and methionine may directly or indirectly alter glucose metabolism in the liver and prevent metabolic disorders. Choline's lipotropic action may prevent hepatic lipidosis, which is credited for limiting gluconeogenesis ([Osorio et al., 2014](#)), and methionine supply may protect the liver from oxidative stress and inflammation that inhibits function; metabolism of both nutrients can biochemically contribute intermediates to the TCA cycle and potentially gluconeogenesis ([Chandler and White, 2019](#))

For aspartate transferase activity (AST), the treatments with BIO decreased AST ( $P \leq 0.05$ ) on treatments that did not receive it. A hepatic protective function has been related to choline consumption. This has been shown from its antioxidant action, lipotropic, in integrity and signaling functions of cell membranes ([Fardet, 2010](#)). Its supplementation has reduced some liver enzymes, indicating an improvement in the hepatic status of dairy cattle ([Rahmani et al., 2014](#)) and the increase of choline-deficient diets in piglets ([Getty and Dilger, 2015](#)), [Sanchez et al. \(2021\)](#) mentioned that AST levels could be attributable to the herbal formulas containing bioactive substances, with a possible role in decrease diseases by the phyto-genic properties. Results allow concluding that the herbal choline and methionine can be improved by supplementing cows with the evaluated herbal sources due to changes in plasmatic glucose and AST.

## NOVELTY STATEMENT

The authors propose using polyherbal mixtures that contain choline and methionine. The results suggest that herbal sources of amino acids influence the productivity of dairy cows and generate some change in plasmatic parameters as AST concentrations.

## AUTHOR'S CONTRIBUTION

Hector A. Lee-Rangel designed the experiment. Fulgencio Ferretiz-Rodriguez, Gregorio Alvarez-Fuentes and Jose Alejandro Roque-Jimenez conducted the experiment and collected samples. Hector A. Lee-Rangel and Juan Carlos Garcia-Lopez analyzed the sample and data. Hector A. Lee-Rangel and Fulgencio Ferretiz-Rodriguez wrote the manuscript. Rolando Rojo-Rubio revised the manuscript.

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.



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