Milk production in dairy cows supplemented with herbal choline and methionine

Producción de leche en vacas suplementadas con colina y metionina herbales

German David Mendoza [1], Mario Francisco Oviedo [2], Juan Manuel Pinos [3], Héctor Aarón Lee-Rangel [2], Anayeli Vázquez [2], Rogelio Flores [4], Francisco Pérez [5], Alejandro Roque [2], Oswaldo Cifuentes [2]

Originales: *Recepción:* 29/11/2017 - Aceptación: 17/10/2018

Abstract

The objective of this study was to evaluate the supplementation of herbal choline and methionine to dairy cows on milk production and milk composition during 60 days of lactation, while also identifying the volatile compounds of the herbal methionine and choline. Fourteen compounds were identified for herbal methionine and fifteen in herbal choline including aromas, alcohols, aldehydes and phenolics, some with nutraceutical properties. Twenty-multiparous Holstein cows (body condition score, BCS = 3.1 ± 0.15; mean ± SE) were fed a basal diet (16.3% CP, 1.6% DP and 1.71. Mcal/kg ME). Seven days after calving, cows were randomly assigned to treatments, which consisted of control basal diet or an oral dose of herbal choline (15 g/d) plus herbal methionine (10 g/d). The experiment lasted 60 days with measurements of milk production and composition every 7 days. Supplementation with herbal choline plus herbal methionine improved (P <0.05) milk production (32.96 *vs.* 34.03 kg/d) and 4% FCM (28.23 *vs.* 29.91 kg/d). Protein content decreased (P <0.05) on supplemented cows (29.9 *vs.* 31.7 g/kg). However, no effects on the remaining composition (fat, lactose, total solids and non-fatty solids) was found. Milk production can be improved by supplementing cows with the evaluated herbal sources of choline and methionine.

Keywords
volatile compounds • herbal • dairy production • choline • methionine

1 Universidad Autónoma Metropolitana. Unidad Xochimilco. 04960 México
2 Universidad Autónoma de San Luis Potosí. Facultad de Agronomia y Veterinaria. San Luis Potosí. 78321. México. hector.lee@uaslp.mx
3 Universidad veracruzana. Facultad de Medicina Veterinaria y Zootecnia. Veracruz. 91710. Mexico.
4 Universidad Autónoma del Estado de México. Centro Universitario UAEM Temascaltepec. Temascaltepec 51300. México.
5 Universidad Autónoma de San Luis Potosí. Centro de Biociencias.

Resumen

El objetivo de este experimento fue evaluar la suplementación con metionina y colina herbales en ganado lechero sobre la producción, cambios en composición de leche durante 60 días de lactancia, e identificar los compuestos fitoquímicos volátiles en metionina y colina herbales. Se identificaron 14 compuestos para metionina y quince para colina herbales entre los que se incluyen aromáticos, alcoholes, aldehídos, fenoles, algunos con propiedades nutracéuticas. Se usaron 20 vacas multíparas raza Holstein (condición corporal CC=3,1 ± 0,15) alimentadas con una dieta basal (16,3% PC, 6% RDP y 2,08 Mcal/kg EM). Siete días después del parto fueron asignadas a uno de los tratamientos que consistían en dieta testigo y la dieta testigo con dosis oral de colina (15 g/d) más metionina (10 g/d) herbales. El experimento tuvo una duración de 60 días, se registró la producción de leche y se caracterizó su composición. La suplementación de colina herbal con metionina herbal mejoró la producción (P<0.05) de leche (32,96 *vs.* 34,03 kg/d) y 4% FCM (28,23 *vs.* 29,91 kg/d), el contenido de proteína disminuyó (P<0,05) (29,9 *vs.* 31,7 g/kg) pero no afectó la composición. Se mejora la producción de leche al suplementar vacas con fuentes naturales de colina y metionina.

Palabras clave
compuestos volátiles • herbal • producción de leche • colina • metionina

Introduction

Herbal additives may improve animal health and production. However, many phytochemical action mechanisms are unknown. In this sense, to correctly identify these phytochemicals and their appropriate doses in order to safely use them is important (15). Many standardized herbal products used in animal feed have not been fully characterized yet.

The use of electronic nose based on different sensor technologies has been suggested as a rapid detection of quality -related volatile compounds for various food products (16, 40) facilitating the identification of nutra- ceutical properties in those products.

The use of ruminally protected choline (RPC) has demonstrated that choline is a limiting nutrient for milk production in dairy cattle (20, 36). Evaluations of herbal products in lambs indicate that some of these products have ruminally protected choline (18) that could be an alternative for dairy cattle. Methionine has been recognized as one of the limiting amino acids for milk production in dairy cows (33) and even when the ruminally protected form is available and has been evaluated (24) the benefit-cost ratio can make its inclusion difficult in some units.

During the transition from pregnancy to lactation, dairy cows present a period of negative energy and protein balance as a result of an increased metabolic demand from the mammary gland (11). Since methyl donors are required for the synthesis of key compounds such as phosphatidylcholine and carnitine in tissues (37), a negative methyl donor balance also may be an important challenge for the transition dairy cow.

Due to extensive microbial degradation in the rumen, dietary availability of key methyl donors is limited (17). Thus, the possibility of improving milk production in dairy cows by increasing the duodenal flow of choline and methionine with herbal products should be evaluated. Some authors report that evaluations for 30 to 90 days are valid to find responses in dairy cows (4, 9, 25, 36, 37). Therefore, the objective of this study was to evaluate the supplementation whith herbal methionine and choline on dairy cows on milk production and milk composition during 60 days of lactation.

Material and methods

Evaluations of volatile organic compounds (VOCs) in herbal choline and methionine by flash gas chromatography electronic nose

The flash gas chromatography electronic nose (FGC- E-Nose) model Heracles II, equipped with an automatic injection unit HS100 (AlphaMOS®, Tolouse, France), was used to detect the VOCs of the herbal choline and methionine.

The Heracles II was equipped with two columns working in parallel mode: a non-polar column (DB-5: 5% phenyl- 95% dimethylpolysiloxane) and DB-1701 (14% cyanopropylphnyl- 86% dimethylpoly-siloxane). The injector was maintained at a constant temperature of 200°C. The samples of the feed plant additives were placed in 20 mL magnetically sealed vials with a plug and without any treatment or extraction solvent. The vials were placed in the Heracles II auto-sampler, which was placed in a shaker oven and shaken at 500 rpm for 900 seconds at 40°C. Next, 1 mL sample was taken from the headspace in the electronic nose. Samples were analyzed in triplicate. A single chromatogram was created by joining two columns of overlapping chromatograms, helping to reduce identification errors. The identifications were made using the Kovats index with a C6-C16 standard (29, 35). The GC subjected the samples to a temperature program separating the volatile organic compounds and maintaining a constant flow of hydrogen of 1 mL/min. Then the samples were brought to a temperature of 50°C for 30 s, before increasing it 10°C / s until it reached 280°C. Separate species were detected by the electronic nose software using multi-variable statistical analysis (Alpha Soft® by Alpha MOS®).

Chemometrics

In this study, a first explorative step was carried out using peak areas that were automatically calculated by the software Alpha Soft® which uses raw data from the abundance of metabolites to construct a multivariate model using Principal Component Analysis (PCA). The PCA uses orthogonal transformation to convert a set of observations by the different compounds of possibly correlated variables into values of linearly uncorre-lated variables. This analysis guaranteed independence if the group of data jointly normally distributed. The PCA is a chemo-metric procedure that rotates the original space to another one and its vectors resul-tants are the principal components (PC) oriented along directions containing the maximum explained variance (29).

Productive phase

The experiment was conducted at the experimental station of the UASLP (22°11' N, 100°56' O, 1850 m above sea level) with a mean temperature of 17.5°C. Twenty multiparous dairy Holstein cows (body condition score, BCS = 3.1 ± 0.15;

mean ± SE), were fed a basal diet (16.3% CP, 6 RDP and 2.08 Mcal/kg ME) of oat hay, alfalfa hay, rolled corn and concentrate (65% forage, 35% concentrate).

Seven days after parturition, the cows were randomly assigned to one of the two treatments (N=10), which consisted in a control group and an oral dose of herbal choline (15 g/d) plus herbal methionine (10 g/d).

To prepare the doses of herbal products, a mixture of 250 g of molasses and 1250 g of corn flour was prepared and stored in the refrigerator at 3°C. Later, a total of 50 g of this mixture was mixed with the daily dose of herbal choline and herbal methionine for each animal, preparing a mixture and individually feeding to ensure its consumption.

The cows received the herbal mixture individually in the milking parlor at 6:00 hour. During the rest of the day, cows had access to a yard with water *ad libitum*.

The herbal products used were OptiMethionine and BioCholine (Technofeed, Mexico, Nuproxa Switzerland, Indian Herbs) supplied individually for 60 days. Milk production was recorded daily and its composition, (fat, protein, lactose, total solids and non-fatty solids) characterized every 7 days using morning and afternoon samples which were mixed, homogenized in a water bath for 1 min (40°C) until the temperature reached 29°C and analyzed with a Lactoscan Ultrasonic milk analyzer (Milkotronic®, Bulgaria). 4% fat corrected milk (FCM) of each cow was calculated as follows: FCM = [(0.4 kg milk) + (0.15 kg milk fat %)].

The yield of energy corrected milk (ECM) was calculated by the formula proposed by DeFrain *et al.* (2006): ECM = [(0.327 kg milk) + (12.95 kg fat) + (7.2 kg protein)]. Body condition score was assessed twice on d 1 and 56 using the scale of 1 to 5, in increments of 0.25 according whit Edmonson *et al.* (1998), at the time of enrollment.

Feed analysis

Samples of feed were composited every 15 days to analyze dry matter and total nitrogen according to the AOAC (1999) (table 1). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyses were carried out according to Van Soest *et al.* (1991).

Table 1. Experimental diet and chemical composition.

Tabla 1. Dieta experimental y composición química.

Item	%
Alfalfa hay	53.45
Oat hay	11.29
Corn rolled	8.06
Concentrate[a]	24.20
Minerals and vitamins[b]	3.0
Chemical composition	
Dry matter (%)	89.73
Crude protein (%)	16.30
Rumen degradable protein (%)[b]	6.0
FDN (%)	34.54
FDA (%)	26.15
ED (Mcal/kg) [c]	2.08

[a] Nu-3® Ganado Lechero18% Línea Campestre: DM 88%, CP 18%, EE 2%, CF 15%, Ash 11.5% and TND 41.5%.

[b] Mineral Premix: Ca 5.6g, vitamin A 500,000 IU, vitamin D 150,000 IU and vitamin E 1000 IU.

[c] Estimated according to the NRC (2001).

[a] Nu-3® Ganado Lechero18% Línea Campestre: MS 88%, PC 18%, EE 2%, FC 15%, Ceniza 11,5% y NDT 41,5%

[b] Premezcla Mineral: Ca 5,6g, vitamina A 500,000 UI, vitamina D 150,000 UI y vitamina E 1000 UI.

[c] Estimado de acuerdo con el NRC (2001).

Statistical analyses

Data were analyzed as a completely randomized design (42). Data were analyzed with the JMP7 software (39) using the General Linear Model.

Results

The Kovat's indices database allowed identification of 14 major relevance compounds in herbal methionine and 15 in herbal choline (table 2 and table 3, page 337) including aromas, alcohols, aldehydes, and phenolics, some with nutraceutical properties.

Figure 1 (page 337), shows that, according whit de PCA, and regarding composition, herbal choline and methionine are totally different (99.84%).

Milk production was significantly increased (3.2%, $P<0.01$). 4% fat corrected milk and yield of energy corrected milk decreased ($P<0.02$). Protein and fat contents decreased. However, no effects on the other milk components were detected (table 2).

Discussion

The electronic nose was used for rapid qualitative detection and discrimination of herbal choline and methionine while a gas chromatography mass spectrometer with headspace analyzer (GCMS-HS) was used for aroma profiling.

The Principal component analysis (PCA) allowed the visualization of the resemblance and difference among the products. OptiMethionine and BioCholine samples were separated in PC1 which described 57.8% of the peak variations (figure 1, page 337).

Table 2. Tentative identification of volatile compounds of herbal methionine from the electronic nose profile.

Tabla 2. Identificación tentativa de los compuestos volátiles de metionina herbal derivados del cromatógrafo de nariz electrónica.

Retention Time, m	Compound	Relative Area	Relative height
Polar Column			
36.81	2-methylbutanoic acid	1.24	1.49
41.38	Alpha-phellandrene	3.9	5.29
45.24	Undecane	12.92	9.37
47.15	[Z]-3-hexenyl isobutyrate	11.33	7.98
58.14	Methyl undecanoate	2.87	3.36
No Polar Column			
15.28	Trimethylamine	3.31	2.35
17.8	Diethyl ether	3.3	3.45
23.35	2,2,4-trimethylpentane	2.59	2.20
32.26	[E]-2-penten-1-ol	1.43	1.65
36.34	2-methylbutanoic acid	3.78	4.57
53.31	Alpha-ionone	23.22	18.43
60.36	Delta-decalactone	2.60	2.48

Table 3. Tentative identification of volatile compounds of herbal choline from the electronic nose profile.

Tabla 3. Identificación tentativa de los compuestos volátiles de colina herbal derivados del cromatógrafo de nariz electrónica.

Retention Time, m	Compound	Realtive Area	Relative height
Polar Column			
18.93	Diethyl ether	4.44	4.61
43.19	[Z]-2-octenal	5.48	6.18
44.53	P-Cresol	2.55	3.27
47.51	4-ethylphenol	33.07	34.64
53.59	4-vinylguaiacol	5.81	7.27
56.14	Trans-2-undecenal	3.97	3.20
No Polar Column			
15.26	Trimethylamine	2.49	2.02
17.27	Diethyl ether	1.50	1.58
21.62	1-propanol	2.69	2.40
24.25	Isopropyl acetate	3.82	3.60
34.90	Beta pinene	4.56	6.91
48.53	Methylnonanedione	5.38	8.10
51.45	4-vinylguaiacol	3.87	3.21
55.38	P-menthadienhydroperoxide	36.91	27.90

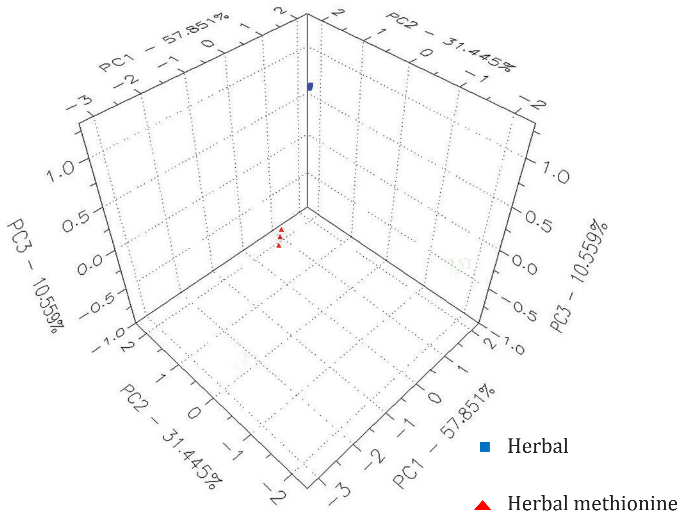

Figure 1. PCA model built with the electronic nose data related to the herbal methionine and herbal choline.

Figura 1. PCA con datos obtenidos del cromatógrafo de nariz electrónica para colina y metionina herbales.

The fact that the herbal products are positive in one axis and negative on the other, is because the metabolites are from different plants with different aromatic profiles, for example BioCholine is elaborated with plans such as *Trachyspermum ammi, Azadirachta indica* and *Andrographis paniculata* whereas OptiMethionine *Trigonella foenumgraceum* and *Allium sativum.*

Milk production, 4% fat corrected milk and yield of energy corrected milk were increased (*P*<0.01). The benefits of supplementing choline and methionine protected from rumen degradation have been reported by several authors including meta-analyses (34, 37). However, none evaluated herbal sources. Several authors have reported that 12 to 15 grams per day of RPC, increase milk production on 7 to 8% (20, 24) representing about 2 kg/d of milk, similar to that observed in this experiment.

Although some experiments have used doses from 25 to 60 g of different RPC sources based on choline chloride (30, 38, 41) or even higher doses (6, 13), Pinotti *et al*. (2005) concluded that the best responses for improved milk production are obtained when 12-20 g/day of RPC are provided which is similar to the dose used with the herbal product.

Regarding ruminally protected methionine (RPM), Lara *et al.* (2006) found that milk production was increased up to 14% above the control with 16 g per day, that later decreased. Similar rresponses were observed in first-calf heifers with doses from 14 to 16 g/d (5).

In both studies, the concentration of milk protein was increased by RPM. Zhou *et al.* (2016b) observed an increment of 9% in milk production and increasing milk protein content with an estimated dose of 14 g/d. Some experiments have supplemented the combination of Met and choline in dairy cattle rations. Zhou *et al.* (2016a) did not find RPC - RPM interactions in any measured variable. Sun *et al.* (2016) reported that both nutrients improved milk production (5.04%) and increased milk fat and protein. In contrast, Soltan *et al.* (2012) observed that milk production increased with both nutrients in a greater magnitude (14%) than with RPC (11%) or RPM (6%) separately, without changes in milk composition. Milk protein content was decreased (P<0.02) by the herbal supplementation however, no effects on the other milk components were detected (table 4, page 339). After methionine supplementation, an average increase of 3.8 kg/d in milk production in the first 30 d of lactation was detected by Zhou *et al.* (2016b). Considering that Met has been identified as one of the 2 most limiting AA for lactating cows (33) and that a greater DMI would increase daily protein intake, the milk yield response when supplementing Met to achieve a Lys: Met close to the suggested optimum, was as expected. The supplementation with rumen-protected methionine improved milk protein synthesis (48), while high percentages of total solids in the choline+methionine group was not associated with milk protein and milk fat percentage in those cows. The supplementation of methionine and/or choline has been evaluated in terms of the contribution to the improved performance and immuno-metabolic status in dairy cows by Zhou *et al.* (2016b) who recognized the predominate effects of Met.

Table 4. Effect of herbal choline and methionine supplementation on milk production and composition of Holstein cows.

Tabla 4. Efecto de la suplementación con colina y metionina herbales en la producción y composición de leche de vacas Holstein.

	Control	Choline + Methionine	SEM	*P*-value
Milk yield (kg/d)	32.96 [b]	34.03 [a]	0.2394	0.01
4% FCM (kg/d)	28.23 [b]	29.91 [a]	0.2321	0.01
ECM (kg/day)	.34 [a]	31.56 [b]	0.2456	0.01
Body Score Condition				
Day 1	3.13	3.08	0.044	0.44
Day 56	2.77	2.69	0.057	0.31
Milk composition (g/kg)				
Fat	33.9	28.3	0.2562	0.51
Protein	31.7 [a]	29.9 [b]	0.039	0.02
NFS	66.0	72.5	0.396	0.28
Lactose	46.1	48.0	0.087	0.17
Total Solids	176.0	180.7	0.423	0.45

NFS: non-fat solids; SEM: standard error of the mean.

NFS: sólidos no grasos; SEM: error estándar de la media.

In contrast, Sun *et al.* (2016) show a correlated response in the blood antioxidant status and in the immune response (plasma interleukin 2, concentration and tCD4+/CD8+ T lymphocyte ratio) in postpartum cows. Other studies have demonstrated the antioxidant capacity of choline *per se* (43, 49) while the supplementation of RPM and RPC has also improved reproductive performance in dairy cows (4).

Considering health risks during the peripartal period (4, 49), methionine and choline should be supplemented around parturition. Methionine supplementation has led to a lower incidence of ketosis (49), besides improving lactation performance. It has also demonstrated greater pre- and postpartum DMI, milk fat yield, and milk protein yield during the peripartal period. However, this has not been consistent with choline supplementation (48).

The herbal choline and methionine containing bioactive compounds (table 2, page 336 and table 3, page 337) may help protect dairy cattle against diseases, maintaining animal health during the critical peripartal period.

Reviewing the properties of the main compounds found in Biocholine and Optimethionine, it may be speculated that herbal products have nutraceutical properties. Andrade *et al.* (2016) reported high cytotoxicity effects of p-menthane and its derivatives against human tumor cells evaluated in mice experimental sarcoma tumors. Hüe *et al.* (2015) reported that the main constitutes of p-menthane are thymol (30.5%) and γ-terpinene (33.0%) with nutraceutical properties.

The 4-vinylguaiacol has demonstrated antioxidant properties in cultured hepatocytes (14), while the β-pine, part of the essential oils of different *Pinus* species, has anti-inflammatory and cytotoxic activity.

The β-pinene is a potential agents for anticancer and anti-inflammatory drugs (7) and it is possible that the aldehyde β-pines monoterpene conformation is responsible for the ruminal protection of the biocholine given that β-pinene has bacteriostatic and bactericidal effects (46).

Lin *et al.* (2013) reported that α-phellandrene promoted immune responses in a murine model, stimulating macrophage proliferation and promoting cell function *in vivo*.

The Undecane has been found as part of the structure of limonoids with cytotoxic activities and anti-inflammatory activity studied in the root barks of *Walsura robusta* (14). The Trans-2-Undecenal, also found in *Citrullus vulgaris* (23) and *Curcuma amada,* is an aldehyde recognized for the characteristic mango aroma (31) that we hypothesized contributed to the ruminal protection of BioCholine. Other compounds in *Mango ginger* have been recognized with some anti-breast cancer effects (27).

Some compounds such as alcohols (table 2, page 336; table 3, page 337) may not be beneficial given that various *in vitro* studies indicate that ethanol may reduce ruminal microbial activity (19). Ethanol was detected in OptiMethionine; nevertheless, mixed ruminal microbes can convert ethanol to carboxylic acids, acetic, butyric and hexanoic (47). Ethanol can be found in the rumen in minor concentrations with barley additions. There are no reports of its enrolment in metabolic diseases in cows (8).

The 1-propanol has antiseptic and disinfectant properties (44) and could be metabolized by ruminal microbes as another alcohol. The 2 Pentanol is an aromatic compound reported in fresh bananas and other foods (22, 32). The amounts of alcohols found in the herbal products should not be a concern, as demonstrated in cow performance.

Conclusion

The herbal choline and methionine contain numerous volatile phytochemical compounds. Milk production can be improved by supplementing cows with the evaluated herbal sources, considering a reduction in the milk protein content but without altering other milk components.

References

1. Andrade, L. N.; Lima, T. C.; Amaral, R. G.; Do, Ó.; Pessoa, C.; De Moraes Filho, M. O.; Soares, B. M.; Do Nascimento, L. G.; Carvalho A. A. C.; De Sousa, D. P. 2015. Evaluation of the cytotoxicity of structurally correlated p-menthane derivatives. Molecules. 20(7): 13264-13280.
2. Andrade, L. N.; Amaral, R. G.; Dória, G. A. A.; Fonseca, C. S.; Da Silva, T. K. M.; Júnior, R. L. C.; Thomazzi, S. M.; Nascimento, L. G. D.; Carvalho, A. A.; De Sousa, D. P. 2016. *In vivo* anti-tumor activity and toxicological evaluations of perillaldehyde 8,9-epoxide, a derivative of perillyl alcohol. International Journal of Molecular Science. 17(1): 32, 2-11.
3. AOAC (Association of Official Analytical Chemists). 1999. Methods of Analysis. Washington D.C. USA.
4. Ardalan, M.; Rezayazdi, K.; Dehghan-Banadaky, M. 2010. Effect of rumen-protected choline and methionine on physiological and metabolic disorders and reproductive indices of dairy cows. Journal of Animal Physiology and Animal Nutrition. 94: 259-265.
5. Ayala, J.; Mendoza, G. D.; Pinos, J. M.; Lara, A.; Sánchez, M. T. 2010. Effects of fat and rumen-protected methionine supplementation on production response of first-lactation cows. Acta Agriculturae Scandinavica. Section A, Animal Science. 60: 159-165.

6. Banevičius, E.; Einorytė, S.; Falkauskas, R. 2016. Choline chloride possible impact on cow´s productivity. Veterinarija ir Zootechnika. 73: 95.

7. Basholli-Salihu, M.; Roswitha, S.; Avni, H.; Dafina, M.; Helmut, V.; Behxhet, M.; Monika, M. 2017. Phytochemical composition, anti-inflammatory activity and cytotoxic effects of essential oils from three *Pinus spp.* Pharmaceutical Biology. 55: 1553-1560.

8. Burim, A.; Qendrim, Z.; Fozia, S. 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics. 6: 583-594.

9. Davidson, S.; Hopkins, B. A.; Odle, J.; Brownie, C.; Fellner, V.; Whitlow, L. W. 2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. Journal of Dairy Science. 91: 1552-1559.

10. DeFrain, J. M.; Hippen, A. R.; Kalscheur, K. F.; Schingoethe, D. J. 2006. Feeding lactose to increase ruminal butyrate and the metabolic status of transition dairy cows. Journal of Dairy Science. 89: 267-276.

11. Drackley, J. K. 1999. ADSA Foundation Scholar Award. Biology of dairy cows during the transition period: The final frontier? Journal of Dairy Science. 82: 2259-2273.

12. Edmonson, A. J.; Lean, I. J.; Weaver, D. L.; Farver, T.; Webster, G. 1998. A body condition-scoring chart for Holstein dairy cows. Journal of Dairy Science. 72: 68-78.

13. Elek, P.; Newbold, J. R.; Gaal, T.; Wagner, L.; Husveth, F. 2008. Effects of rumen-protected choline supplementation on milk production and choline supply of periparturient dairy cows. Animal. 2: 1595-1601.

14. Esatbeyoglu, T.; Ulbrich, K.; Rehberg, C.; Rohn, S.; Rimbach, G. 2015. Thermal stability, antioxidant, and anti-inflammatory activity of curcumin and its degradation product 4-vinyl guaiacol. Food and Function. 6: 887-893.

15. Frankič, T.; Voljč, M.; Salobir, J.; Rezar, V. 2009. Use of herbs and spices and their extracts in animal nutrition. Acta agriculturae Slovenica. 94: 95-102.

16. Gardner, J. W.; Bartlett, P. N. 1999. Electronic noses. Principles and applications. Oxford University Press: Oxford. England.

17. Girard, C. L.; Matte J. J. 2005. Folic acid and vitamin B12 requirements of dairy cows: A concept to be revised. Livestock Production Science. 98: 123-133.

18. Godinez-Cruz, J.; Cifuentes-López, O.; Cayetano, J.; Lee-Rangel, H. A.; Mendoza, G.; Vázquez, A.; Roque, A. 2015. Effect of choline inclusion on lamb performance and meat characteristics. Journal of Animal Science. 93 (Suppl. 3) 766 (Abstr).

19. Gomes, M. F. F.; Ítavo, C. C.; Ítavo, L. C.; Leal, C. R.; Silva, J. A.; Heimbach, N. S.; Leal, E. S. 2017. *In vitro* fermentation characteristics of ruminant diets using ethanol extract of brown propolis as a nutritional additive. Revista Brasileira Zootecnia. 46(7): 599-605.

20. Hartwell, J. R.; Cecava, M. J.; Donkin, S. S. 2000. Impact of dietary rumen undegradable protein and rumen-protected choline on intake, peripartum liver triacylglyceride, plasma metabolites and milk production in transition dairy cows. Journal Dairy Science. 83: 2907-2917.

21. Hüe, T.; Cauquil, L.; Fokou, J. B. H.; Dongmo, P. M. J.; Bakarnga-Via, I.; Menut, C. 2015. Acaricidal activity of five essential oils of *Ocimum* species on *Rhipicephalus (Boophilus) microplus* larvae. Parasitoogyl Research. 114(1): 91-99.

22. Jordan, M. J.; Tandon, K. S.; Philip, E.; Goodner, K. L. 2001. Aromatic profile of aqueous banana essence and banana fruit by Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Olfactometry (GC-O). Journal Agricultural Food Chemistry. 49(10): 4813-4817.

23. Kemp, T. R. 1975. Identification of some volatile compounds from *Citrullus vulgaris*. Phytochemistry. 14(12): 2637-2638.

24. Lara, A.; Mendoza, G. D.; Landois, L. A.; Bárcena, R.; Sánchez, T.; Rojo, R.; Ayala, J.; Vega, S. 2006. Milk production in Holstein cows supplemented with different levels of ruminally protected methionine. Livestock Science. 105: 105-108.

25. Leiva, T.; Cooke, R.; Brandao, A. 2015. Efects of rumen protected choline supplementation on metabolic and performance responses of transition dairy cows. Journal of Animal Science. 93: 1896-1904.
26. Li, Y.; Li, S.; Meng, X.; Gan, R. Y.; Zhang, J. J.; Li, H. B. 2017. Dietary natural products for prevention and treatment of breast cancer. Nutrients. doi: 10.3390/nu9070728.
27. Lima, F. S.; Sá Filho, M. F.; Greco, L. F.; Santos, J. E. P. 2012. Effects of feeding rumen-protected choline on incidence of diseases and reproduction of dairy cows. Veterinary Journal. 193: 140-145.
28. Lin, J. J.; Lin, J. H.; Hsu, S. C.; Weng, S. W.; Huang, Y. P.; Tang, N. Y.; Lin, J. G.; Chung, J. G. 2013. Alpha-phellandrene promotes immune responses in normal mice through enhancing macrophage phagocytosis and natural killer cell activities. *In Vivo.* 27(6): 809-14.
29. Melucci, D. A.; Bendini, F.; Tesini, S.; Barbieri, A.; Zappi, S.; Vichi, T.; Toschi, G. 2016. Rapid direct analysis to discriminate geographic origin of extra virgin olive oils by flash gas chromatography electronic nose and chemometrics. Food chemistry. 204: 263-273.
30. Munafo, J. P.; Didzbalis, J.; Schnell, R. J.; Schieberle, P.; Steinhaus, M. 2014. Characterization of the major aroma-active compounds in mango (*Mangifera indica L.*) cultivars haden white alfonso, praya sowoy, royal special, and malindi by application of a comparative aroma extract dilution analysis. 2014, Journal Agricultural of Food Chemistry. 62(20): 4544-4551.
31. Nogueira, J. M. F.; Fernandes, P. J. P.; Nascimento, A. M. D. 2003. Composition of volatiles of banana cultivars from Madeira Island. Phytochemical Analysis. 14: 87-90.
32. NRC. 2001. Nutrient Requirements of Dairy Cattle. National Research Council. The National Academies Press, Washington. DC. USA.
33. Patton, R. A. 2015. Effect of rumen-protected methionine on feed intake, milk production, true milk protein concentration, and true milk protein yield, and the factors that influence these effects: A meta-analysis. Journal of Dairy Science. 93: 2105-2118.
34. Peng, Q.; Tian, R.; Chen, F.; Li, B.; Gao, H. 2015. Discrimination of producing area of Chinese Tongshan kaoliang spirit using electronic nose sensing characteristics combined with the chemometrics methods. Food chemistry. 178: 301-305.
35. Piepenbrink, M. S.; Overton, T. R. 2003. Liver metabolism and production of cows fed increasing amounts of rumen-protected choline during the periparturient period. Journal of Dairy Science. 86: 1722-1733.
36. Pinotti, L.; Baldi, A.; Dell'Orto V. 2002. Comparative mammalian choline metabolism with emphasis on the high-yielding dairy cow. Nutrition Research Reviwes. 15: 315-332.
37. Pinotti, L.; Campagnoli, A.; Dell'Orto, V.; Baldi, A. 2005. Choline: Is there a need in the lactating dairy cow? Livestock Production Science. 98: 149-152.
38. Sales, J.; Homolka, P.; Koukolova, V. 2010. Effect of dietary rumen-protected choline on milk production of dairy cows: A meta-analysis. Journal of Dairy Science. 93: 3746-3754.
39. Sall, J.; Lehman, A.; Stephens, M.; Creighton, L. 2012. JMP® Start Statistics: A Guide to Statistics and Data Analysis. Cary. NC. USA: SAS Institute In.
40. Soltan, M. A.; Mujalli, A. M.; Mandour, M. A.; El-Shinway Abeer, M. 2012. Effect of dietary rumen protected methionine and/or choline supplementation on rumen fermentation characteristics and productive performance of early lactating cows. Pakistan Journal Nutrition. 11: 221-230.
41. Steel, G. D. R.; Torrie, J. H.; Dickey, D. A. 1997. Principles and Procedures of Statistics. A Biometrical Approach. New York: McGraw-Hill.
42. 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.
43. Tattanahalli, L.; Nagabhushan. 1980. 1-Aryl-2-acylamido-3-fluoro-1-propanols, methods for their use as antibacterial agents and compositions useful therefore. Patent US4235892.
44. Uribe, S.; Ramirez, J.; Peña, A. 1985. Effects of beta-pinene on yeast membrane functions. Journal of Bacteriology. 161: 1195-1200.

45. Van Soest, P. J.; Robertson, J. B.; Lewis, B. A. 1991. Methods for dietary fibre, neutral detergent fibre, and nonstarch carbohydrates in relation to animal nutrition. Journal of Dairy Science. 74: 3583-3597.
46. Weimer, P. J.; Kohn, R. A. 2016. Impacts of ruminal microorganisms on the production of fuels: how can we intercede from the outside?. Applied Microbiology and Biotechnology. 100: 3389-3398.
47. Zhou, Z.; Bulgari, O.; Vailati-Riboni, M.; Trevisi, E.; Ballou, M. A.; Cardoso, F. C.; Luchini, D. N.; Loor, J. J. 2016a. Rumen-protected methionine compared with rumen-protected choline improves immunometabolic status in dairy cows during the peripartal period. Journal of Dairy Science. 99: 8956-8969.
48. Zhou, Z.; Vailati-Riboni, M.; Trevisi, E.; Drackley, J. K.; Luchini, D. N.; Loor, J. 2016b. Better postpartal performance in dairy cows supplemented with rumen-protected methionine compared with choline during the peripartal period. Journal of Dairy Science. 99: 8716-8732.
49. Zhu, J.; Wu, Y.; Tang, Q.; Leng, Y.; Cai, W. 2010. The effects of choline on hepatic lipid metabolism, mitochondrial function and antioxidative status in human hepatic C3A cells exposed to excessive energy substrates. Nutrients. 6: 2552-2571.

Acknowledgement

Authors thank financial support to FAI-UASLP C18-FAI-05-34.34.
The authors acknowledge Nuproxa México for donating the herbal product and Eng. Martin Castillo for technical assistance.