Response of LH and Progesterone in Postpartum Cows Added with Different Levels of Protected Methionine

Alejandro Lara[1], Germán David Mendoza[2], Teresa Sánchez-Torres[3], Pedro Abel Hernández[4], José Antonio Martínez[2]*

[1] Universidad Autónoma Chapingo, km 38.5 Carretera México-Texcoco, Chapingo, Edo. México, 56230, México.
[2] Universidad Autónoma Metropolitana, Unidad Xochimilco, Departamento de Producción Agrícola y Animal, Coyoacán 04960, México.
[3] Colegio de Postgraduados, Montecillo Edo. de México 35340, México.
[4] Universidad Autónoma del Estado de México. Centro Universitario Amecameca. Amecameca, 56900, México.
* jamarg@correo.xoc.uam.mx

Abstract: The objectives of this study were to analyze to assess the progesterone (P_4) and LH concentrations in response to doses of protected methionine (0, 8, 16, and 24 g d^{-1}) in postpartum dairy cows. A total of 12 cows were used, assigned (n = 3) to each of the doses. From day 15 to day 96 postpartum (four normal estrous cycles), 10 ml of blood was extracted from the jugular vein of each cow to determine concentrations of P_4, whereas LH samples were obtained on days 14 and 21 of each of the four cycles with an interval of 6 hours 15 min per sample (10:00 to 16:00). The results for the first three cycles showed higher P_4 concentrations ($P < 0.05$) for the treatment with 16 and 8 and 24 g d^{-1}; whereas for the last cycle, there were no differences ($P > 0.10$) as a consequence, accumulated P4 showed differences higher ($P < 0.05$) concentration in cows fed 8 and 24 g d-1. The LH concentration or peaks were not affected ($P > 0.10$) by protected methionine. It was concluded that at doses of 8 and 24 g d-1 of protected methionine increases accumulated P4 during the first 75 days post-partum which appears to improve the function of the corpus luteum without effect on LH.

[Lara A, Mendoza GD, Sánchez-Torres T, Hernández PA, Martínez JA. **Response of LH and Progesterone in Postpartum Cows Added with Different Levels of Protected Methionine.** *Life Sci J* 2015;12(2s):104-107]. (ISSN:1097-8135). http://www.lifesciencesite.com. 16

Keywords: LH, Methionine, Postpartum cows, Progesterone

1. Introduction

Increasing milk production in modern dairy farms is associated with reduced fertility and reproductive performance (Lucy *et al.*, 2001) which is related to the negative energy balance (NEB), which is apparently the most important factor in the early lactation (Stevenson, 2001). Dairy cows with high genetic merit for milk production usually show longer intervals to first postpartum ovulation and take longer time to display a normal luteal function compared with cows with lower genetic potential (Garverick and Smith, 1986). The failure in the function of the corpus luteum (CL) that causes reduced fertility has been considered the result of selection for milk production (Garverick, 1997). One strategy to counteract the negative effects of NEB on it reproductive behavior is to increase the energy density of the diet (Garnsworthy *et al.*, 2008), supplementing dietary fat that reduces the duration of NEB, and increases the size of the ovulatory follicle and lifespan of the CL of dairy cows (Mattos *et al.*, 2000); and in beef cows, increase concentrations and peaks of LH (Funston, 2004).

Also the protected methionine of ruminal degradation has been used in dairy cows to improve the yield and composition of milk (Lara *et al.*, 2006). In grazing heifers, under tropical conditions, methionine supplementation before the breeding season improved body condition and ovulation rate (Alonso *et al.*, 2008), indicating that the availability of methionine may improve the ovarian function. Therefore, the aim of this study was to evaluate whether methionine improves the concentration P_4, peaks and circulating LH in postpartum high producing cows.

2. Material and Methods

The study was conducted at the Experimental Farm of Universidad Autónoma Chapingo, in the State of Mexico, Mexico. A total of 12 multiparous cows, average live weight (606 ± 21.3 kg) with 2-5 lactations and between 25 and 30 kg of milk production in lactating previous, which were clinically healthy, were used following the guidelines established by the Law Animal Protection of the State of Mexico, México (1985). Animals were supplemented for 96 days postpartum with four (n = 3) different levels (0, 8, 16 and 24 g d^{-1}) of protected methionine of ruminal degradation (RPMet; Mepron®M85, Degussa-Hülls), with the aim of evaluating the effects of methionine levels on P_4 and LH at the start of the postpartum period.

The cows were fed a basal diet consisting of alfalfa hay, corn silage and concentrate, with 48%

forage and 52% concentrate, containing 19.6% CP, 35% RUP, and 1.7 Mcal kg^{-1} NEm. The cows were randomized to one of four treatments (levels of protected methionine). The response variables were P_4 concentrations, cumulative P_4, number of peaks and LH concentrations.

From day 15 postpartum, cows declared clinically and reproductively healthy were selected for the study when a level of over 1 ng mL^{-1} of P_4 was observed for five consecutive days. Starting on this day were four periods of 21 days, simulating normal estrous cycles; P_4 concentrations were measured every third day, using 10 ml of blood obtained by venipuncture. Subsequently, the samples were centrifuged for serum separation at 3,500 rpm for 15 min (centrifuged Beckman J2-HS, USA) within the first hour after collection, and were kept refrigerated at -20°C until they were analyzed to determine P4 concentrations by radioimmunoassay (Coat-A-Count, Diagnostic Product Corp., Los Angeles, CA, USA). The intraassay coefficient of variation was 8.5%. Cumulative P_4 was also determined for the periods evaluated.

About 10 ml of blood was taken from each cow by venipuncture for LH concentrations to determine, starting from 15 d postpartum; they were assessed on days 14 and 21 of each of the four periods for P_4, in 6 hours interval with a difference of 15 min per sample (10:00 to 16:00 h). The samples were centrifuged for serum separation at 3,500 rpm for 15 min in a maximum period of 1 h post-collection; serum samples were kept refrigerated at -20°C until analyzed for LH (Niswender *et al.*, 1968) by radioimmunoassay (Coat-A-Count, Diagnostic Product Corp., Los Angeles, CA, USA). The sensitivity of detection was 0.1 ng ml^{-1} and coefficients of variation between and inter trials were 8 and 10%, respectively. Also, LH pulse was defined when the LH level was higher than the value of one standard deviation above the overall mean of the sampling periods (McDowell *et al.*, 1998).

The mean concentrations of LH and P_4 were compared by Tukey's test (Steel *et al.*, 1997). The P_4 concentration and the accumulated were tested by homogeneity regression after adjusting a polynomial regression by day (Wilcox *et al.*, 1990). All analyses were performed using the Statistical Analysis System (SAS, 2001).

3. Results

In the first three estrous cycles there were difference among treatments in progesterone (Table 1) with the lowest concentration with 16 g d^{-1}; whereas in the last cycle, there was no effect of the methionine ($P > 0.10$), indicating that the effects of the addition of methionine only affect P_4 during the first 75 days postpartum. As expected from the final P_4 accumulated concentration showed higher values ($P < 0.05$) in treatments with 24 g (319.20a ng mL^{-1}) and 8 g (291.73a ng mL^{-1}), followed by control (188.11b ng mL^{-1}) and the 16 g (125.98b ng mL^{-1}) of ruminally protected methionine.

Table 1. Effect of inclusion of protected methionine (0, 8, 16 and 24 g d-1) dose on the daily production of progesterone (ng mL^{-1}) per cycle in early postpartum of dairy cows

Estrous cycle	Protected methionine levels, g d^{-1}				
	0	8	16	24	SEM
1	1.44^{ab}	2.75^{a}	0.82^{b}	3.06^{a}	0.46
2	1.73^{ab}	2.63^{a}	1.01^{b}	2.96^{a}	0.36
3	1.84^{ab}	2.27^{ab}	1.14^{b}	2.92^{a}	0.38
4	1.40	2.30	1.37	1.87	0.44

a,b Literals distinct within rows are different ($P < 0.05$).
SEM standard error of the mean.

Regarding to LH concentrations (Table 2), during the first interval, the treatment with 24 g d^{-1} methionine showed a higher concentration ($P < 0.05$) than the rest of the treatments. In contrast, in the intervals 2, 4 and 6, differences were observed between treatment with 16 g d^{-1} methionine, which was the highest concentration compared to other treatments; during third interval there was no difference between treatments ($P > 0.10$), whereas in fifth interval, the control treatment showed a higher average concentration compared to other treatments. Only in this interval dose of 16 g d^{-1} methionine had the lower concentration, not finding a physiological explanation for this data.

Table 2. Effect of inclusion of protected methionine (0, 8, 16 and 24 g d^{-1}) dose on the LH levels (ng mL^{-1}) in six hours periods in early postpartum of dairy cows

	Protected methionine levels, g d^{-1}				
Interval*	0	8	16	24	SEM
1	1.38^{a}	1.42^{a}	1.76^{a}	4.07^{b}	0.41
2	1.17^{a}	1.70^{a}	6.31^{b}	2.77^{a}	0.70
3	1.69	1.80	1.93	1.33	0.16
4	1.70^{a}	1.21^{a}	2.51^{b}	1.70^{a}	0.17
5	8.68^{a}	2.56^{b}	1.70^{b}	2.10^{b}	0.70
6	1.63^{a}	1.72^{a}	3.21^{b}	1.49^{a}	0.26
7	2.83^{a}	2.82^{a}	5.93^{b}	1.05^{a}	0.54
8	13.9^{a}	12.5^{a}	1.75^{b}	2.01^{b}	1.70

a,b Literals distinct within rows are different ($P < 0.05$).
*Period of 6 h with an interval of 15 min per sample (10:00 to 16:00 h).
SEM standard error of the mean

The number of LH peaks observed during the intervals (Table 3) were similar among treatments ($P > 0.10$) between each intervals evaluated there was no response to the dose of methionine.

Table 3. Effect of inclusion of protected methionine (0, 8, 16 and 24 g d^{-1}) dose on number of LH peaks by week in early postpartum dairy cows.

	Protected methionine levels, g d^{-1}				
Week	0	8	16	24	SEM
1	7.0	7.3	6.0	6.6	0.55
2	6.3	6.0	7.3	7.0	0.47
3	7.3	6.3	7.3	7.3	0.33
4	6.6	6.6	6.0	6.3	0.57
5	7.0	7.0	5.6	7.0	0.60
6	6.6	7.6	6.6	7.0	0.40
7	7.0	6.0	7.3	8.0	0.66
8	7.3	6.3	7.0	7.3	0.57

4. Discussion

The effects of protected methionine on P4 concentrations have not been previously reported. Butler *et al*. (2006) indicated that when animals are in negative energy balance showed a decrease in serum P4 concentrations and fertility, in this condition, methionine addition increase the amount of this amino acid available for the functioning of the corpus luteum; however, essential amino acid supplementation has shown inconsistent results on reproductive performance in dairy cows (Roche, 2006). If dietary protein levels are high then negative results can be observed, diets with 19 and 20% protein can increase the number of days to first estrus, and reduced plasma concentration of progesterone to 50% (Butler, 2003).

It has been reported that increasing the non-degradable protein content in the diet from 11.1 to 15.7% reduces CL development and maximum plasma concentration of progesterone in dairy cows; these negative effects can be avoided by supplementing protein with 2.2% fat protected ruminal degradation (Garcia-Bojalil *et al*., 1998). In contrast, in beef cows, increasing metabolizable protein restarts luteal activity, reducing the days from calving to first estrus (Waterman *et al*., 2006).

Increasing dietary energy improves the function of CL in dairy cows in NEB (Leroy *et al*., 2010) and methionine has a role in energy metabolism. Methionine can be converted into succinyl CoA and can enter the Krebs cycle for energy production (Nelson *et al*., 2008). Normally, methionine is an important metabolite in the hepatic metabolism of lipids, having an effect on carnitine synthesis that affects the transport of fatty acids into the mitochondria for metabolism by β oxidation (Piepenbrink *et al*., 2004). Thus, it presumably methionine improves the utilization of fatty acids to provide energy at the tissue level which could had an impact on the function of CL, affecting increased P4 production.

Piepenbrink *et al*. (2004) indicated that the use methionine in dairy cows tends to reduce NEB. The hormonal response to the amino acid is also affected by the production level of the cow; milk production shows a quadratic response to protected methionine in dairy cows with the higher production with 16 g d-1 producing 2 kg d^{-1} more of milk in relation to lower or higher doses (Lara *et al*., 2006).

Cows with NEB mobilize muscle protein and protected methionine may reduce the amount of this amino acid from the tissue. The methionine methyl groups participate in a large number of transmethylation reactions involved in the regulation of the activity of DNA and sulfur groups in the synthesis of cysteine (Harpaz, 2005). Studies with rats fed with a ketogenic diet may provide an additional hypothesis to explain the effects of methionine, methionine might preserve the lean mass in cows and may also increase the expression of fatty acid oxidation genes (Pissios *et al*., 2013). It has been demonstrated that protected methionine increase milk protein content (Lara *et al*., 2006).

The lack of response in LH to protected methionine is consistent with results reported by Rusche *et al*. (1993) for primiparous beef cows between 3 and 35 days postpartum, fed 100 or 150% of recommendations for crude protein CP in diets that contained low escape protein or high escape protein and did not observe changes in the amplitude, frequency and mean LH concentration. In dairy cows, increasing rumen degradable protein has been found to reduce conception rates, probably because it tends to reduce the NEB and result in a greater loss in body condition, which can affect the normal development of LH secretion (Westwood *et al*., 2000).

Increased progesterone concentrations and methionine supplementation suggests a better quality in the corpus luteum at doses of 8 and 24 g d-1 of protected methionine, effects which manifested only during the first 75 days postpartum. No changes were detected in LH peaks.

Corresponding Author:
Dr. José Antonio Martinez García
Departmento de Producción Agrícola y Animal
Universidad Autonoma Metropolitana Xochimilco
Mexico Distrito Federal, 04960 México
E-mail: jamgar@correo.xoc.uam.mx

References

1. Lucy MC. Reproductive loss in high-producing dairy cattle: where will it end?. J. Dairy Sci. 2001, 84: 1277-1293.
2. Stevenson JS. Reproductive management of dairy cows in high milk-producing herds. J. Dairy Sci. 2001, 84: E128-E143.

3. Garverick AH, Smith SM. Mechanisms associates with subnormal luteal function. J. Anim. Sci. 1986, 62(Suppl. 2): 92-105.
4. Garverick AH. Ovarian follicular cysts in dairy cows. J. Dairy Sci. 1997, 80: 995-1004.
5. Garnsworthy PC, Lock A, Mann GE, Sinclair KD, Webb R. Nutrition, metabolism, and fertility in dairy cows: 1. Dietary energy source and ovarian function. J. Dairy Sci. 2008, 91: 3814-3823.
6. Mattos R, Staples CR, Thatcher WW. Effects of dietary fatty acids on reproduction in ruminants. Rev. Reprod. 2000, 5: 38-45.
7. Funston, R. N. Fat supplementation and reproduction in beef females. J. Anim. Sci. 2004, 82: E154–E161.
8. Lara A, Mendoza GD, Landois LA, Bárcena R, Sanchez-Torres, Rojo R, Ayala J, Vega S. Milk production in Holstein cows supplemented with different levels of ruminally protected methionine. Livest. Prod. Sci. 2006, 105: 105-108.
9. Alonso L, Maquivar M, Galina CS, Mendoza GD, Guzmán A, Estrada S, Villareal M, molina R. Effect of ruminally protected methionine on the productive and reproductive performance of grazing *Bos indicus* heifers raised in the humid tropics of Costa Rica. Trop. Anim. Health Prod. 2008, 40: 667-672.
10. Ley de Protección Animal del Estado de México. Published in Gaceta del Gobierno del Estado de México September 4, 1985. Naucalpan Estado de México, México.
11. Niswender GD, Reichert LE, Midgley AR, Nalbandov AV. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 1968, 84: 1166-1173.
12. McDowell CM, Anderson LH, Kinder JE, Day ML. Duration of treatment with progesterone and regression of persistent ovarian follicles in cattle. J. Anim. Sci. 1998, 76: 850-855.
13. Steel RGD, Torrie JH, David A, Dickey DA. Principles and procedures of statistics: A biometrical approach. 2ª ed. NY, US: McGraw-Hill Book Co. 1997, pp. 666.
14. Wilcox CJ, Thatcher WW, Martin FG. Statistical analysis of repeated measurements in physiology experiments. 1990, P 141. In: Proc. Final Research Co-ordination Meeting of the FAO/IAEA/ARCAL III Regional network for Improving of the Reproduction Management of Meat- and Milk-Producing Livestock in Latin America with the Aid of Radioimmunoassay International Atomic Energy Agency. Vienna Austria. Meeting-of-the-Arcal-III-Programme-Bogota-19-23-Sep-1988.
15. SAS. Statistical Analysis System SAS/STAT® User's Guide (Release 6.03). SAS Inst. Inc. Cary, N.C. USA. 2001.
16. Butler ST, Pelton SH, Butler WR. Energy balance, metabolic status, and the first postpartum ovarian follicle wave in cows administered propylene glycol. J. Dairy Sci. 2006, 89: 2938–2951.
17. Roche JF. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim. Reprod. Sci. 2006, 96: 282-296.
18. Butler WR. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest. Prod. Sci. 2003, 83: 211–218.
19. Garcia-Bojalil CM, Staples CR, Risco CA, Savio JD, Thatcher WW. Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows: productive responses. J. Dairy Sci. 1998, 81: 1385-1395.
20. Waterman, RC, Sawyer JE, Mathis CP, Hawkins DE, Donart GB, Petersen MK. Effects of supplements that contain increasing amounts of metabolizable protein with or without Ca-propionate salt on postpartum interval and nutrient partitioning in young beef cows. J. Anim. Sci. 2006, 84: 433-446.
21. Leroy JLMR, Langbeen A, Van Hoeck V, Bols PEJ. Effect of energy balance on oocyte and embryo quality in modern dairy cows. Advan. Dairy Technol. 2010, 22: 71-79.
22. Nelson DL, Lehninger AL, Cox MM. Lehninger Principles of Biochemistry. Cap. 16. Krebs Cylcle. Editor Freeman. USA. 2008.
23. Piepenbrink MS, Marr AL, Waldron MR, Butler WR, Overton TR, Vázquez-Añón M, Holt MD. Feeding 2-hydroxy-4-(methylthio)-butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. J. Dairy Sci. 2004, 87: 1071-1084.
24. Harpaz S, 2005. L-carnitine and its attributed functions in fish culture and nutrition. A review. Aquaculture 2005, 249: 3-21.
25. Pissios P, Hong S, Kennedy AR, Prasad D, Liu FF, Maratos-Flier E. 2013. Methionine and choline regulate the metabolic phenotype of a ketogenic diet. Mol. Metab. 2013, 2: 306–313.
26. Rusche WC, Cochran RC, Corah LR, Stevenson JS, Harmon DL, Brandt Jr RT, Minton JE. Influence of source and amount of dietary protein on performance, blood metabolites, and reproductive function of primiparous beef cows. J. Anim. Sci. 1993, 71: 557-563.
27. Westwood CT, Lean IJ, Garvin JK, Wynn PC. Effects of genetic merit and varying dietary protein degradability on lactating dairy cows. J. Dairy Sci. 2000, 83: 2926-2940.

2/11/2015