

## LECITHIN AS A SUPPLEMENT FOR MID-LACTATING DAIRY COWS

Roberta DE NARDI <sup>1</sup>, Giorgio MARCHESINI <sup>1</sup>, Sandro TENTI <sup>1</sup>, Barbara CONTIERO <sup>1</sup>, Igino ANDRIGHETTO <sup>2</sup>, Severino SEGATO <sup>1</sup>

### ABSTRACT

The study aimed at evaluating the effect of a daily dose of 14 g of choline administered by using soy lecithins (LC group), a by-product of the biodiesel production process, compared to a daily dose of 25 g of choline chloride microencapsulated with hydrogenated vegetable oils (RPC group) on performance and milk quality of dairy cows. A total of 12 mid-lactating Holstein Friesian cows were assigned to one of two experimental groups and fed according to a cross-over design (2 diets × 2 periods) one of the two isoenergetic, isofibrous and isonitrogenous diets, characterized by LC or RPC choline supplement. Dry matter intake, milk yield and the 3.5% fat-corrected milk (FCM) were not affected by the diet. No differences were observed in the hematological profile or milk composition, with the exception of NEFA and milk fat, which resulted significantly higher and lower ( $P < 0.10$ ) in LC-fed cows, respectively. Summarizing, results indicated that soy lecithins can be used as an available source of choline in mid lactating dairy cows, even at a daily dose lower than that required when using RPC.

**Key words:** cattle / dairy cows / animal nutrition / lecithin / choline / fat corrected milk

### 1 INTRODUCTION

Rumen-protected choline (RPC) supplementation to transition and early lactating dairy cows was demonstrated to improve not only milk production (Erdman and Sharma, 1991; Pinotti *et al.*, 2003), but also lipid synthesis (Piepenbrink and Overton, 2003). Pinotti *et al.* (2002) reported that choline may improve the transport of lipids in the blood, thus reducing the risk for fatty liver disease and ketosis in periparturient dairy cows. However many studies showed that choline chloride fed to ruminants is extensively degraded in the rumen (Sharma and Erdman, 1989; Bonomi *et al.*, 1996), and for this reason, dietary unprotected choline seemed to contribute insignificantly to the choline body pool. As an alternative to the use of RPC, crude lecithins could be a cost

effective sources of choline since they contain around 20% of phosphotidylcholine and other phosphatides (i.e., phosphatidylethanolamine and phosphatidylinositol), and lecithins are obtained as a by-product of the water degumming process in the biodiesel production (Van Gerpen, 2004). Comparing a similar supplementation of choline (a daily dose of around 25 g per cow) as lecithins or RPC in mid-lactating dairy cows, Marchesini *et al.* (2012) did not find any differences in performance and milk quality, except for the reduction of milk fat content. Therefore, the aim of this study was to test the effect of a lower dose of lecithins on dry matter intake (DMI), metabolic profile, milk yield, milk quality traits and milk fatty acid composition of mid-lactating dairy cows.

<sup>1</sup> Dept of Medicine Animal, Production and Health Padova Univ., Agripolis, Viale dell'Università, 16, 35020 Legnaro (PD), Italy

<sup>2</sup> Istituto Zooprofilattico delle Venezie, Viale dell'Università, 10, 35020 Legnaro (PD), Italy

## 2 MATERIALS AND METHODS

### 2.1 DIETARY TREATMENTS AND EXPERIMENTAL DESIGN

The Padova University Animal Care and Use Committee approval was obtained before the commencement of the study. According to a cross-over design (2 diets  $\times$  2 periods), 12 Holstein Friesian cows were grouped in two experimental groups balanced for milk yield ( $35.3 \pm 6.0$  kg/d), days in milk ( $160 \pm 35$  d) and parity ( $2.3 \pm 1.6$ ). Each experimental period lasted 21 days (14 days of adjustment phase followed by 7 days of data collection). The two dietary treatments differed for the supplements added to the TMR diet (based on maize silage): one based on soy lecithins (LC group) derived from biodiesel production process, the other based on choline chloride in a rumen-protected form (RPC group). Lecithins (35% of DM) had 3.8% of choline, meanwhile RPC (91% of DM) had 25% of choline chloride coated by hydrogenated vegetable oils. Dietary treatments were formulated to be isoenergetic, isonitrogenous and isofibrous; diet ingredients and composition are presented in Table 1.

**Table 1:** Formulation ( $\text{g}\cdot\text{kg}^{-1}$  of diet on DM) and proximate composition (% of DM) of experimental diets

Diet	LC	RPC
Supplemented choline, g/cow	14	25
Maize silage	378	384
Cereal mix	182	185
Soybean blend	167	168
Permanent meadow and alfalfa hay	204	205
Beet dry pulps and crushed linseed	53	54
RPC	-	4.3
Lecithin	6.0	-
Crude protein	14.3	14.4
Ether extract	5.0	4.9
Crude ash	7.0	7.1
NDF	30.7	30.8
Non-fibre carbohydrates	43.0	42.8

According to a hypothetical DMI of 23.0 kg/d, mean supplemented choline contents available daily to each cow were 14 and 25 g for LC- and RPC-diet, respectively. Cows were housed in a free stall, in two different pens, were fed *ad libitum* at 0900 h and milked twice a day with an automated milking plant. Feed intake was individually and continuously recorded through an automated feeding control system (Biocontrol A/S, Rakkestad, Norway).

### 2.2 PERFORMANCE, CHEMICAL ANALYSIS AND STATISTICAL EVALUATION

Cows were milked twice a day and milk yield was manually recorded during the final 5 days of each period. A milk sample, containing morning and afternoon milk at a 1:1 ratio was collected twice, on the 2<sup>nd</sup> and on the 5<sup>th</sup> day of each experimental week; milk samples were analysed for fat, protein and lactose using a MilkoScan FT plus (Fossomatic, Foss electric, Hillerød, 113 Denmark). The urea content was determined using differential pH-metry method (EUROCHEM CL 10 plus, Microlab EFA). For the determination of the milk FA composition, samples were stored at  $-80$  °C until analyses. FA composition was determined after lipid extraction by chromatography, using a dichloromethane/methanol solution (2:1 v/v). Aliquots of the extracts were then trans-esterified and fatty acid methyl esters (FAME) were detected as described by Egger *et al.* (2007). Before morning milking, jugular vein blood samples were taken twice during each experimental week. The samples were collected into heparinised tubes and centrifuged to obtain plasma. The plasma was analysed for haematological parameters by using a biochemical auto-analyser (Hitachi 912, Roche Diagnostics GmbH Mannheim), except for non-esterified fatty acids (NEFA) which was assessment to a manual procedure (Novatech Diagnostics, Randox).

After verifying the normality and variance homogeneity (PROC UNIVARIATE and Shapiro-Wilk test), a mixed model procedure (PROC MIXED) was performed to evaluate data on feed intake, milk and 3.5% FCM yield, metabolic profile, milk quality and fatty acid composition. The linear random model included the fixed effects of dietary treatment and period along with their interaction, the random effect of cow and the random residual. All statistical analyses were carried out by SAS (2008).

**Table 2:** Effect of lecithine on performance and milk quality

Diet	LC	RPC		
Supplemented choline, g/cow	14	25	P	SEM
DMI, kg/d	21.4	22.2	ns	0.9
Choline intake, g/d	13.0	24.1	***	0.8
Milk production, kg/d	33.7	33.8	ns	1.6
FCM 3.5%, kg/d	32.2	33.0	ns	1.5
Milk quality				
Crude protein, %	3.10	3.09	ns	0.07
Crude fat, %	3.26	3.42	†	0.14
Lactose, %	4.88	4.89	ns	0.06
Urea, mg/dL	23.8	23.9	ns	0.8

†:  $P < 0.1$ ; \*\*\*:  $P < 0.001$ ; ns: not significant

**Table 3:** Effect of lecithin on haematological profile

Diet	LC	RPC	P	SEM
Supplemented choline, g/cow	14	25		
Total protein, g/L	78.2	78.9	ns	1.1
Albumin, g/L	35.0	34.6	ns	0.4
Globulin, g/L	43.3	44.3	ns	1.0
AST, U/I	98.2	94.4	ns	2.8
$\gamma$ GT, U/I	25.2	25.8	ns	0.9
CK, U/I	228	195	ns	25
Urea, mmol/L	4.47	4.36	ns	0.17
Glucose, mmol/L	3.58	3.55	ns	0.07
Triglycerides, mmol/L	0.12	0.13	ns	0.01
NEFA, mmol/L	0.16	0.13	*	0.01
Total cholesterol, mmol/L	5.16	5.20	ns	0.41
$\beta$ -hydroxybutyrate, mmol/L	0.56	0.55	ns	0.04
NEFA/Cholesterol ratio	0.033	0.026	*	0.004

\*:  $P < 0.05$ ; ns: not significant

### 3 RESULTS AND DISCUSSION

As reported in Table 2, no differences between the two groups in DMI, milk yield and 3.5% FCM were observed, possibly because diets had similar composition and the degree of choline protection against ruminal degradation resulted the same. Both experimental groups showed a slightly lower daily choline intake (13.0 and 24.1 g/d) if compared to the amount calculated according to a DMI of 23.0 kg. Milk urea and milk composition did not differ between groups, except for crude fat ( $P < 0.1$ ) (Table 2).

**Table 4:** Effect of lecithin on fatty acid (FA) composition of milk (% of the total detected FA)

Diet	LC	RPC	P	SEM
Supplemented choline, g/cow	14	25		
C18:0	12.0	11.7	ns	0.4
C18:1 n-9	22.9	22.8	ns	0.5
C18:1 n-7	2.9	2.8	ns	0.2
C18:2 n-6	4.4	4.4	ns	0.2
C18:3 n-3	0.85	0.84	ns	0.03
CLA	0.91	0.96	ns	0.04
Saturated FA	64.7	64.4	ns	0.8
Monounsaturated FA	28.6	28.3	ns	0.6
Polyunsaturated FA	7.0	7.1	ns	0.2

ns: not significant

These findings are in agreement with the previous study of Marchesini *et al.* (2012) who found a similar level of the milk choline content between the two choline sources, suggesting a similar post-ruminal choline bioavailability. The same milk yield and 3.5% FCM between the two theses, despite LC-diet had a lower level of choline, could be justified by a diversified metabolic route of choline derived from lecithins or RPC, as confirmed by the lower level of milk fat in LC fed cows. Likely the 14 g of choline from LC-diet were enough to cover the extra demand for methyl groups in mid-lactating dairy cows (Pinotti *et al.*, 2002), supporting the milk production, but gave a lower contribution to milk fat. Choline in fact plays a major role in lipid transport because phosphatidylcholine is an essential component of the very low density lipoproteins (VLDL) produced in the liver and cannot be substituted by other phospholipids. As the long-chain FA in milk are obtained from the blood triacylglycerols of VLDL, which arise from absorbed fat and, endogenously via the mobilisation of adipose fat stores, a different metabolic use of the phosphatidylcholine present in lecithins, could have reduced the VLDL formation and led to a lower milk fat content.

No statistical differences in the blood parameters were found between the diets, except for NEFA and NEFA/cholesterol ratio that were higher in LC-diet fed cows (Table 3). NEFA and NEFA to cholesterol ratio are indicators of the fat retained in or metabolized by the liver, thus their higher values could suggest a less efficient liver function and lipid metabolism in LC group (Piepenbrink and Overton, 2003). However their levels in this study were very low if compared to those relieved by Pinotti *et al.* (2003), and together all the other parameters they fell within the physiological range of lactating dairy cows (Cozzi *et al.*, 2011).

No statistical differences in milk FA composition were detected between dietary treatment (Table 4). In fact, despite LC showed a higher content of unsaturated fatty acids (UFA) than RPC (LC: 60.7% of EE on DM basis of which 78.4% of UFA; RPC: 51.0% of EE on DM basis of which 0.3% of UFA), the biohy-

drogenation process of the UFA in the rumen resulted in the same milk acidic profile (Marchesini *et al.*, 2009).

#### 4 CONCLUSIONS

The results of this study suggested that soy lecithins derived by the biodiesel production process can be used as an available source of choline in mid-lactating dairy cows. A daily dose of around 14 g/cow allowed a similar milk yield and quality in comparison with a group of dairy cows receiving a dose of 25 g of microencapsulated choline chloride per daily in a period after first 2-months of lactation. However, the use of LC-diet led to a slightly lower milk fat, even though fatty acid composition was not affected. Further investigation is required to determine how different forms of choline could influence choline bioavailability for the different metabolic, especially in dairy cows in early lactation to verify whether lecithins could prevent or alleviate hepatic lipid accumulation.

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#### 6 REFERENCES

- Bonomi A., Quarantelli A., Bonomi B.M., Sabbioni B., Superchi P. 1996. L' integrazione delle razioni per le bovine da latte con colina in forma rumino-protetta. Effetti sull'efficienza produttiva e riproduttiva (Inclusion of rumen-protected choline in diets for dairy cattle. Effect on productive and reproductive efficiency). *Rivista Scienze Alimentari*, 25: 413-434
- Cozzi G., Ravarotto L., Gottardo F., Stefani A.L., Contiero B., Moro L., Brscic M., Dalvit P. 2011. Reference values for blood parameters in Holstein dairy cows. Effects of parity, stage of lactation, and season of production. *Journal of Dairy Science*, 94: 3895-3901
- Egger P., Holzer G., Segato S., Werth E., Schwienbacher F., Peratoner G., Andrighetto I., Kasal A. 2007. Effect of oilseed supplements on milk production and quality in dairy cows fed a hay-based diet. *Italian Journal of Animal Science*, 6: 227-239
- Erdman R.A., Sharma B.K. 1991. Effect of dietary rumen-protected choline in lactating dairy cows. *Journal of Dairy Science*, 74: 1641-1647
- Marchesini G., Andrighetto I., Stefani A., Berzaghi P., Tenti S., Segato S. 2009. Effect of unsaturated fatty acid supplementation on performance and milk fatty acid profile in dairy cows fed a high fibre diet. *Italian Journal of Animal Science*, 8: 391-403
- Marchesini G., Segato S., Stefani A.L., Tenti S., Dorigo M., Gerardi G., Bernardini D., Andrighetto I. 2012. Lecithin: a by-product of biodiesel production and a source of choline for dairy cows. *Italian Journal of Animal Science*, *in press*
- 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
- Pinotti L., Baldi A., Dell'Orto V. 2002. Comparative mammalian choline metabolism with emphasis on role in ruminants, especially the high yielding dairy cow. *Nutrition Research Reviews*, 15: 315-331
- Pinotti L., Baldi A., Politis I., Rebucci R., Sangalli L., Dell'Orto V. 2003. Rumen protected choline administration to transition cows: effects on milk production and vitamin E status. *Journal of Veterinary Medicine. A, Physiology, Pathology, Clinical Medicine*, 50, 1: 18-21
- SAS. 2008. User's Guide. Version 9.2. Cary, NC, USA, SAS Institute Inc.
- Sharma B.K., Erdman R.A. 1989. Effects of dietary and abomasally infused choline on milk production responses of lactating dairy cows. *Journal of Nutrition*, 119: 248-254