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## PALMITIC ACID INCREASES MILK FAT CONTENT AND YIELD IN LACTATING COWS<sup>1</sup>

# [EL ÁCIDO PALMÍTICO INCREMENTA LA CONCENTRACIÓN Y LA PRODUCCIÓN DE GRASA LÁCTEA EN VACAS LACTANTES]

C. Tódero, E. Ticiani, W. B. Júnior, E. D. Silva, D. J. Miqueluti and D. E. Oliveira\*

<sup>1</sup> Santa Catarina State University, Av. Luiz de Camões, 2090 - Conta Dinheiro, 89520-000, Lages, SC, Brazil. Phone number: (55) 49-32899224, e-mail: dimas.oliveira@udesc.br

\*Corresponding author

#### **SUMMARY**

Milk fat is the component responsible for the organoleptic and manufacturing characteristics of the dairy products and also the most variable and affected by the diet. This study was designed to evaluate the lipid supplementation with high concentration of palmitic acid on the milk production and composition. Thirty-nine multiparous Holstein cows weighting an average of 550 kg, with  $79 \pm 2$  days in milk (DIM), producing 29.4 kg milk/day were randomly assigned to each one of the three treatments: 1) 320 g/day of high palmitic acid supplement (Palmitic); 2) 400 g/day of calcium salts of fatty acids rich in linoleic acid (Linoleic) and; 3) Control (no lipid supplementation). Palmitic supplement increased milk fat content in 8.6% (P=0.001) and yield 16.2% (P=0.001) compared to Control. Compared to Control, Linoleic supplement decreased milk fat content in 16.1% (P=0.001). There was an effect of treatment for bonus payment for fat (P=0.01), with the highest payment for Palmitic treatment. There was no effect on milk production and concentrations and yields for milk protein and lactose.

**Key words**: Lipids; Lipid Synthesis; Lipid Supplementation.

#### RESUMEN

La grasa láctea es el componente responsable de las propiedades organolépticas y de manufactura de los productos lecheros, y es al mismo tiempo el más variable en función de la dieta. Este estudio fue diseñado para evaluar los efectos de la suplementación lipídica, utilizando ácido palmítico, sobre la producción y la composición de la leche. Treinta y nueve vacas Holstein multíparas, con un peso promedio de 550 kg, a 79 ± 2 días en leche, produciendo 29.4 kg de leche/día, fueron asignadas aleatoriamente a uno de tres tratamientos: 1) 320 g/día de un suplemento enriquecido en ácido palmítico (Palmítico); 2) 400 g/día de sales de calcio de ácidos grasos con alto contenido de ácido linoleico (Linoleico); y 3) Control (sin suplementación lipídica). Comparado con el Control, el suplemento de ácido palmítico incrementó el contenido de grasa láctea en 8.6% y la producción de grasa en 16.2% (P = 0.001), mientras que el suplemento de ácido linoleico redujo el contenido de grasa en 16.1% (P = 0.001). Hubo un efecto de tratamiento sobre el pago de bonificación por grasa (P = 0.01), siendo el más alto para el Palmítico. No hubo efectos sobre la producción de leche, ni sobre la concentración o producción de proteína y lactosa.

Palabras clave: Lipidos; síntesis de lípidos; suplementación de lípidos.

<sup>&</sup>lt;sup>1</sup> Submitted August 27, 2016 – Accepted October 25, 2016. This work is licensed under a <u>Creative Commons Attribution 4.0 International License</u>

#### INTRODUCTION

Breeding programs for increasing genetic merit for milk production are an important strategy for increasing milk yield and milk components, but the energy requirements can be also increased. Meeting the energy requirements has been an important challenge to dairy nutritionists trying to mantain milk production therefore, the use of supplemental fat is a nutritional tool to minimize the negative energy balance and give support to a high milk production. However, the fat source used as a supplement (oilseeds, calcium salts, vegetable oils, fat supplements highly enriched with individual fatty acids) can have diferente responses because they have diferent fatty acid profiles.

Milk fat content and yield are often increased when fat supplements are fed, mainly those made with saturated fatty acid as palmitic (C16:0) and stearic (C18:0) acids (MOSLEY et al., 2007) and that can be an important issue as milk fat represent the main energetic cost for the cows (BAUMAN and GRIINARI, 2001) and may be advantageous economically for producers. The main objective of this study was to evaluate a high-palmitic (88% of total fatty acids) fat supplement fed to dairy lactating cows on milk production and composition and also on the economic viability.

## MATERIALS AND METHODS

All procedures were approved by the Ethical Comitee (protocol n. 01.37.14) of Santa Catarina State University. Thirty-nine Holstein earlylactation multiparous cows (79  $\pm$  2 DIM), weighing  $550 \pm 45$  kg, initial BCS of 2.75 (scale of 1 to 5),  $29.3 \pm 1.6$  kg milk/d were randomly assigned to one of the following treatments (n = 13/treatment): a) Control: no lipid supplement; b) Palmitic: 320 g/d of high-palmitic fat supplement (Wawasan Tebrau Agrolipds Sdn. Bhd, Plo Pasir Gudang, Johor, Malaysia) and; c) Linoleic: 400 g/d (Megalac-E, Church & Dwight, Nova Ponte, MG, Brasil). The palmitic-enriched supplement was in a free fatty acid form, with 88% of C16:0 and approximately 90% of saturated fatty acids (Table 1). All lipid supplements provided the same amount of fat.

The experimental period lasted 40 d with 10 d of adaptation to the experimental routine and 30 d of measurements. The diet was fed as a TMR (Table 2), divided in 3 equal portions and fed 3 times a day

and was formulated to provide the nutrient requirements according to NRC (2001)

Table 1. Fatty acid composition of lipid supplements

	Supplement	
g/100g total fatty acids	Linoleic1	Palmitic <sup>2</sup>
C4:0 to C11:0	0.33	0.18
C12:0	2.85	0.15
C14:0	1.12	0.91
C14:1 cis9	0.01	$nd^3$
C15:0	0.06	0.18
C16:0	14.95	88.12
C16:1 cis9	0.21	nd
C18:0	3.94	2.35
C18:1 <i>cis</i> 9	15.16	2.90
C18:1cis11 to cis13	3.63	nd
C18:2 cis9 cis-12	42.16	0.25
C18:3 cis9,cis12,cis15	3.22	0.15
C20	0.23	0.05
C20:2cis11	0.20	0,01
Unidentified	11.93	4.75

<sup>1</sup>Linoleic: MegalacE, Church & Dwight, Nova Ponte, MG, Brasil; <sup>2</sup>Palmitic: high-palmitic acid (88%), Wawasan Tebrau Agrolipds Sdn. Bhd, Plo Pasir Gudang, Johor, Malaysia; <sup>3</sup>nd = not detected.

Through the experiment all animals were in a freestall system with free acces to water and a mineral salt. Cows were milked twice daily at 0600 and 1800 h and milk yield was recorded automatically (GEA Farm Technologies do Brasil, Jaguariúna, São Paulo, Brasil). Milk samples were collected every 3 d with a preservative (bronopol tablet, D&F Control System, San Ramon, CA, USA) and stored at 4°C before being analyzed for milk components. Dry matter intake (DMI) was recorded by treatment group during the last 5 d of measurements according the difference between offered and orts. Samples of the TMR were taken weekly and composite for chemical analyzes according to AOAC (2000) for DM, CP and the TDN of feeds was calculated according to NRC (2001). Milk components (fat, protein, lactose, and total solids) were determined using infrared analysis (AOAC, 2000) and SCC by flow citometry. The fatty acids profile of high-palmitic and Megalac-E was determined using the procedures and operating conditions of gas chromatography described by Baldin et al. (2013).

Table 2. Chemical composition, amounts and cost of each feed on TMR

Ingredients	Corn silage	Comercial feed <sup>1</sup>	Sorghum sudanense	Yeast <sup>2</sup>	Vitamin/mineral mix <sup>3</sup>
DM (kg/d)	6.0	7.6	4.5	0.03	0.15
Cost (R\$/kg)	0.10	0.82	0.08	15.90	1.90
Composition					
DM (%)	27.1	89.4	90.6	92.2	
Ash (% DM)	4.0	9.5	11.3		
CP (% DM)	8.9	18.4	10.6	30.3	
EE (% DM)	5.2	4.5	2.7		
NDF (% DM)	47.2	21.2	74.5		
ADF (% DM)	24.0	8.3	40.5		
TDN (%)	72.6	79.4	51.8		

<sup>&</sup>lt;sup>1</sup> Cooperativa Agroindustrial Alfa (Chapecó, SC, Brasil); <sup>2</sup> Lallemand (Levucell Sc Farm); <sup>3</sup>Composition: Ca 200 g/kg; P 60 g/kg; S 20 g/kg; Mg 20 g/kg; K 35 g/kg; Na 70 g/kg; Co 15 mg/kg; Cu 700 mg/kg; Cr 10 mg/kg; Fe 700 mg/kg; I 40 mg/kg; Mn 1.600 mg/kg; Se 19 mg/kg; Zn 2.500 mg/kg; Vit. A 200.000 UI/kg; Vit. D3 50.000 UI/kg; Vit. E 1.500 UI/kg (Cia Zootécnica Agrária, Mairinque, SP, Brasil). DM: dry matter; CP: crude protein; EE: ether extract; NDF: neutral detergente fiber; ADF: acid detergente fiber; TDN: total digestible nutrient.

The economic analysis for each different supplement was carried out accounting for the change in milk fat content using the producer bonus or penalty for milk fat according the current payment table from Dairy Partners Americas (DPA/Nestlé/Brasil, 2015available https://www.produtornestle.com.br/servico-Nestleprodutor/pagamento-de-leite.aspx). The inputs for milk bonus calculation were: milk yield (kg) and milk fat (%) multiplied by the payment table. The imputs for supplement costs were: the cost of fat supplement (R\$/kg), amount included in the diet (%) and DMI. The economic viability (EV) was: (bonus/penalty) - (diet cost of supplement); Linoleic suplement cost = U\$ 1.69/kg; Palmitic supplement cost = R\$ 2.11/kg.

#### **Statistical Analysis**

Data were analyzed by MIXED procedure of SAS statistical package (SAS Inst. Inc., Cary, NC). DMI, milk yield, milk components and SCC were analyzed as repeated measures, with the least-square means representing the average of all 30 treatment days and the measure at d 0 used as a covariate (removed when not significant). The model included the fixed effects of day of measurement, treatment and the day of measurement x treatment interaction (removed when not significant). Cow was included as a random effect. Data points with Studentized Residuals outside of  $\pm$  2.5 were considered outliers and excluded from analysis. The Compound Symmetry was the used as the covariance structure,

and chosen according the lowest Akaike information. Treatment effects declared significant at P<0.05 and trends at P<0.10. To analyze the frequency of the distribution of how many days received bonus or were in penalties according milk fat content, data were arc-sine transformed and was applied Tukey test at P<0.05. Reported data are back-transformed.

### RESULTS

All cows remained in good health and consumed the lipid supplements completely. There was a treatment effect on milk fat, as Palmitic increased by 8.3 and 25.8% its concentration when compared to Control and Linoleic, respectively (Table 3). Compared to Control, Linoleic supplementation reduced milk fat content by16.1%. Milk fat yield was increased in 16.2 and 26.6% when compared Palmitic treatment with Control and Linoleic, respectively. The milk yield, milk protein content and yield and lactose content and yield did not differ among the treatments but the total solids was decreased in 5.9% for Linoleic treatment. Compared to Control and Palmitic, the animals on the Linoleic treatment consumed, respectively, 4.1 and 2.7% more feed (Table 3).

The fat supplements had different impacts on the economic viability. The Palmitic supplement increased the bonus by 56% when compared to Control. On the other hand, Linoleic caused a penalty of more than 600% compared to Control (Table 4).

Table 3. Performance and milk composition.

	Treatments				
Variable	Control	Linoleic	Palmitic	SEM	P
Milk yield (kg)	27.6	30.4	29.5	1.43	0.400
Fat (%)	3.6 <sup>b</sup>	3.1°	$3.9^{a}$	0.05	0.001
Fat yield (g)	1002 <sup>b</sup>	919 <sup>b</sup>	1164ª	0.05	0.008
Protein (%)	2.72	2.75	2.74	0.05	0.950
Protein yield (g)	736	844	807	0.03	0.102
Lactose (%)	4.5	4.4	4.4	0.05	0.518
Lactose yield (g)	1226	1332	1032	0,06	0,499
Total solids (%)	11.8 <sup>a</sup>	11.1 <sup>b</sup>	11.9 <sup>a</sup>	0.11	0.001
Total solids yield (kg)	3.2	3.4	3.5	0.16	0.299
Feed intake (kg/DM)*	189.7 <sup>b</sup>	197.5 <sup>a</sup>	192.3 <sup>b</sup>	1.61	0.016

<sup>\* (</sup>Per group of treatment)

Table 4. Financial compensation according milk production and fat composition.

Variable	Control	Linoleic	Palmitic
Milk yield (mean, kg)	27.6	30.4	29.5
Base price (R\$/kg)	0.99	0.99	0.99
Fat (%)	3.6	3.1	3.9
Mean bonus or penalty $(R\$)^*$	$0.50^{b}$	-0.03°	$0.78^{a}$
Fat suplemente cost (R\$/d/cow)	0.00	2.00	2.21
Final return (R\$/milk yield) <sup>1</sup>	13.80	-0.91	23.01
Final milk price (R\$/kg of milk)	1.49	0.96	1.77

<sup>\*</sup>DPA Nestlé Brasil, 2015; ¹(milk yield x mean bonus or penalty); Means followed by different letters are significant different (Control vs. Linoleic, P=0,0001; Control vs. Palmitic, P=0,004; Palmitic vs. Linoleic, P=0,0001); 1US\$ = R\$ 3.272.

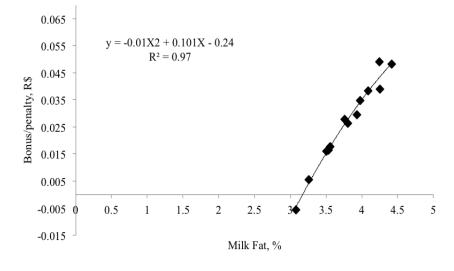
The Figure 1 shows the relationship between milk fat content and the bonus/penalty for milk fat composition during the evaluation period for each treatment.

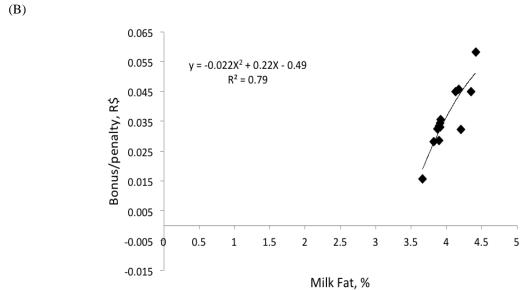
#### DISCUSSION

Studies showing the effects of fat supplements rich in specific fatty acids such as palmitic acid (C16:0) are of interest given that they are more inert in the rumen, producing less bioactive compounds from the biohydrogenation. In this study the animals fed with palmitic acid increased milk fat concentration and yield compared with both others treatments. Interestingly, the Linoleic treatment decreased milk fat content in 13.9 and 20.5%, respectively, compared to Control and Palmitic (Table 3) with a direct impact on the final economic return and milk price (Table 4).

Different responses palmitic to supplementation have been shown where some studies showed positive effects on milk fat (Mosley et al., 2007; Piantoni et al., 2013; Rico et al., 2014a) and others not (Rico et al., 2014b). Recently Rico et al. (2014a) fed a high-palmitic (97.9% of C16:0) and a high-stearic (97.4% of C18:0) to lactating dairy cows and found that high-palmitic increased milk fat content and yield in 3.1 and 5.7%, respectively when compared to supplementation. Lock et al. (2013) fed lactating cows with a high-palmitic supplement (85% of C16:0) and showed increases of 7.2 and 7.3% for milk fat content and yield, respectively. Similar results were shown by Piantoni et al. (2013) feeding a 99% palmitic supplement with 3.3 and 3.5% increasing milk fat concentration and yield as compared to control (soybean hulls).

(A)





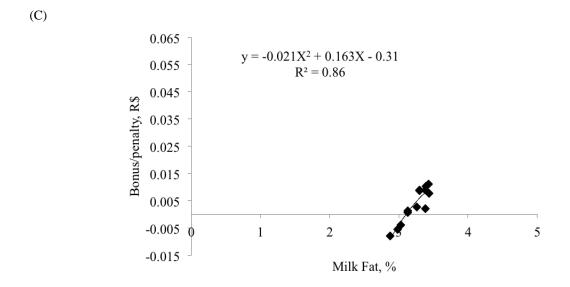


Figure 1. Effects of milk fat concentration on bonus/penalty for Control (A), Palmitic (B) and Linoleic (C) treatments. Data are means of eight analysis from 13 cows in each treatment.

Although we were not able to analyze the milk fatty acid profile and according the BCS of our cows (2.75), and the cited studies, the changes in milk fat content and yield are accounted by the increases in 16 carbon fatty acids in milk. Corroborating that, Rico et al. (2014a) found 24% more milk fat secreted originated from a rich-palmitic supplement (97.9% of C16:0). As described by Thompson & Christie (1991) the increase in milk fat by palmitic acid can be explained because the mammary gland can easily uptake saturated fatty acids (C16:0 > C14:0) from circulation. Reinforcing suggestion, Kinsella & Gross (1973) pointed out that palmitic acid was the main fatty acid acilated on sn-1 position during triglyceride synthesis and Rico et al. (2014a) showed an increase (9.2%) of circulating free fatty acids in blood plasma in cows receiving palmitic acid (C16:0) compared to stearic acid (C18:0), increasing the availability of preformed palmitic acid for uptake by mammary gland, and increasing milk fat concentration and yield.

Comparing unsaturated saturated and fat supplements, the Linoleic supplement used in our study contained mainly C18:2, C18:1 and C18:3 and as a calcium salt it is less inert in the rumen as described by Palmquist & Sukhija (1990) being more able to generate bioactive intermediates during the biohydrogenation causing milk fat depression (MFD). In this study, a plausible explanation for the Linoleic treatment producing less milk fat, is that linoleic acid (C18:2 cis-12, cis-15 - Table 1) from the soybean oil, could dissociate in the rumen, producing biohydrogenation intermediates such as trans-10, cis-12 conjugated linoleic acid (CLA) (Bauman & Griinari, 2003). This fatty acid reduces the gene expression of lipogenic enzymes in the mammary gland, causing MFD (Baumgard et al., 2002).

As showed at Table 4, Palmitic treatment had a better result on milk fat with a higher mean bonus compared to Control and Linoleic. The animals fed with Palmitic received the bonus all the time compared to animals on Control or Linoleic fat supplements. Even though Palmitic treatment increases milk fat content and yield, increasing the bonus for milk fat, its inclusion on the diets will depend of its cost. The inclusion of Palmitic in the diet costs R\$ 2.21, but its income through fat bonus is R\$ 0.78. Interestingly, the Linoleic treatment caused a mean penalty of R\$ 0.03/kg of milk produced.

## **CONCLUSION**

Feeding a high-palmitic acid supplement increased milk fat content and yield with no effects on milk yield and others milk components. The Linoleic supplement caused a milk fat depression with penalty for milk fat payment. The benefits of an inclusion of a high-palmitic supplement it is dependent of its cost and the price system for milk components.

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