

Activity of Δ^9 -desaturase enzyme in mammary gland of lactating buffaloes

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ABSTRACT: The objective of this research was to measure the activity of Δ^9 -desaturase enzyme in lactating buffaloes. Data from forty lactating Murrah-crossbred buffaloes were collected on five commercial farms located at Sarapui and Pilar do Sul, Sao Paulo-Brazil. A field survey was done from April to November 2002. In four farms, buffaloes were fed with wet brewers grains (primary concentrate). Only one farm (Farm 4) offered pasture and corn silage. Monthly milk samples were collected and stored at -20°C until analyzed for fatty acid composition. The Δ^9 -desaturase activity was measured using an indirect method (myristoleic and myristic acids ration - $C_{14:1c9}/C_{14:0}$). The higher $C_{14:1c9}/C_{14:0}$ rate was verified on Farm 4 (0.092). The $C_{14:1c9}/C_{14:0}$ ratio were 0.064 to Farm 1; 0.065 to Farm 2; 0.062 to Farm 3 and 0.065 to Farm 5. The $C_{17:1}/C_{17:0}$, $C_{18:1c9}/C_{18:0}$ and $C_{18:2c9t11}/C_{18:1t11}$ ratios were also affected. The Farm 4 showed higher value for all ratios. Therefore, in lactating buffaloes grazing pasture the Δ^9 -desaturase activity could be enhanced.

Key words: Δ^9 -desaturase, Fatty acid, Pasture, Murrah.

INTRODUCTION - The mammary gland is the major site for Δ^9 -desaturase activity in lactating animals. This enzyme converts specific medium and long chain saturated and polyunsaturated fatty acids that arrive at this gland. Among these acids, the conjugated linoleic acids (CLA) are a group of linoleic acid isomers containing double bonds ($C_{18:2c9t11}$). CLA has been shown for its anticarcinogenic effect. (Bauman *et al.*, 1999). Also, the Δ^9 -desaturase enzyme converts $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$ and $C_{18:1t11}$ into $C_{14:1}$, $C_{16:1}$, $C_{17:1}$, $C_{18:1}$ and $C_{18:2c9t11}$, respectively. $C_{14:1}$ concentration in the digesta is a trace; however, in milk this concentration is high. According to Fievez *et al.* (2003) the myristoleic and myristic acids ration - $C_{14:1c9}/C_{14:0}$ represent a desaturase index and serve as a proxy for Δ^9 -desaturase activity. Diet rich in CLA reduce the Δ^9 -desaturase activity. This occurs due the high

availability of CLA (C18:2c9t11) in rumen which arrive preformed in the mammary gland. The CLA pools presented in milk originate from endogenous (Δ^9 -desaturase) and ruminal sources (Kay *et al.*, 2004). These authors suggest that the Δ^9 -desaturase activity becomes important in pasture grazing animals. Pastures have higher content of linolenic acid (C_{18:3}) which in rumen biohydrogenation process it doesn't produce the C_{18:2c9t11} isomer. Consequently, the rumen concentration of C_{18:1t11} isomer is increased. The trans-11 18:1 will be used by Δ^9 -desaturase as a CLA precursor in mammary gland. The objective of this trial was to measure the activity of Δ^9 -desaturase enzyme using the ratios among C_{14:1c9}/C_{14:0}, C_{16:1c9}/C_{16:0}, C_{17:1}/C_{17:0}, C_{18:1c9}/C_{18:0}, C_{18:2c9t11}/C_{18:1t11} in lactating buffaloes.

MATERIAL AND METHODS - This trial was carried out on a commercial farm located at Sarapui (23°38'28" S and 47°49'38" W) and Pilar do Sul (23°48'44" S and 47°42'29" W) cities, São Paulo-Brazil. In each farm, milk samples of eight buffaloes were collected from April to November 2002. Milk samples were stored at -20° until analyzed for fatty acids composition. The nutritional management adopted in each farm was: Farm 1- buffaloes were maintained in feedlot and fed with corn silage as the main forage source and the wet brewers grain as the primary concentrate; Farm 2 - buffaloes were maintained in feedlot and fed with corn silage either chopped fresh grass (*Pennisetum purpureum*) or sugarcane (*Saccharum officinarum*) and the wet brewers grain as the primary concentrate; Farm 3- buffaloes were maintained on pasture (*Brachiaria decumbens*) supplemented with sugarcane and wet brewers grain as the primary concentrate, Farm 4- buffaloes were maintained on pasture (*Brachiaria decumbens*) supplemented with corn silage and wet brewers grain only in April, October and November; Farm 5- buffaloes were maintained on pasture (*Brachiaria ruziziensis*) supplemented with *B. ruziziensis* silage and concentrate (whole cottonseed, citric pulp and urea) plus wet brewers grain. Only Farm 5 provide total mixed ration to meet the buffaloes requirements. Milk fat from samples was extracted according to Hara & Radim (1978) and fatty acids methylations were prepared as described by Christie (1982) with modifications (Chouinard *et al.* 1999). Fatty acids methyl esters were quantified using a gas chromatograph (Trace GC3). CRM-164 reference standard (Commission of the European Communities, Community Bureau of Reference, Brussels, Belgium) was used to determine recoveries and correction factors for each fatty acid. Data analysis was done by descriptive way due to different systems of animal production.

RESULTS AND CONCLUSIONS - The activity of Δ^9 -desaturase enzyme in mammary gland is measured using an indirect method (myristoleic and myristic acids ratio - C_{14:1c9}/C_{14:0}). In bovines, the average values of C_{14:1c9}/C_{14:0} ratio is 0.062 (Lock & Garnsworthy, 2003; Fievez *et al.*, 2003). The average value found in our experiment (0.069) was similar to the authors' mentioned above (Table 1).

C_{16:1c9}/C_{16:0} ratio determined on all farms was lower (0.054) than published by Lock & Garnsworthy (2003) that found 0,079 to C_{16:1c9}/C_{16:0} ratio. The C_{17:1}/C_{17:0} ratio varied between 0.30 to 0.33 on four of the five farms. However, the average value observed on Farm 4 was 0.48. Higher C_{18:1c9}/C_{18:0} ratio (2.132) was observed in all farms when compared to results reported by Lock & Garnsworthy (2003) that found on average of 1.52. However, the C_{18:2c9t11}/C_{18:1t11} ratio was similar (0.386) to the results published for these authors (0.304), except to the Farm 4 that was increased (0.52). Farms 1, 2, 3 and 5 fed buffaloes with wet brewers grains (rich in linoleic acid). The higher supply of preformed CLA (C_{18:2c9t11}) from

rumen, probably, inhibited its formation in mammary gland from the C_{18:1t11} precursor. Thus, the desaturation index shown in these farms (0.37; 0.36; 0.35 and 0.33; respectively) was 29 to 37% lower than the one observed on Farm 4 (0.52). This supposed inhibition of Δ^9 -desaturase is consistent with the higher relations observed between the products and substrate of this enzyme, previously mentioned. Among the five studied farms, Farm 4 presented the high fatty acids ratio. These results suggest the major activity of the Δ^9 -desaturase enzyme in the buffaloes mammary gland of this farm. According to Kay *et al.* (2004), the contribution of Δ^9 -desaturase in unsaturated fatty acids pool is greater on grazing than confined animals. Similarly, the buffaloes of Farm 4 were maintained on pasture during all experimental period. Therefore, it seems concern to associate the higher Δ^9 -desaturase activity with the diet that the animals received. In conclusion, these result indicated that the Δ^9 -desaturase activity was greater on pasture grazing buffaloes.

Table. 1. Indirect determination of the Δ^9 -desaturase activity in mammary gland of lactating buffaloes.

Ratios	Farm 1	Farm 2	Farm 3	Farm 4	Farm 5
C _{14:1c9} /C _{14:0}	0.064	0.065	0.062	0.092	0.065
C _{16:1c9} /C _{16:0}	0.050	0.051	0.056	0.063	0.051
C _{17:1} /C _{17:0}	0.32	0.30	0.33	0.48	0.31
C _{18:1c9} /C _{18:0}	1.96	1.94	1.98	2.78	2.00
C _{18:2c9t11} /C _{18:1t11}	0.37	0.36	0.35	0.52	0.33

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REFERENCES - Bauman, D. E.; Baumgard, L. H.; Corl, B. A. 1999. Biosynthesis of conjugated linoleic acid in ruminants. In: American Society Of Animal Science. Ithaca. Proceedings, Ithaca: Cornell University, 1-15. Chouinard, P.Y.; Corneau, L.; Barbano, D. M. 1999. Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows. *Journal of Nutrition*, 129:8, 1579-1584. Christie, W. W. 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol ester. *Journal of Lipids Research*, 23:1072. Fernandes, S. A. A.; Mattos, W. R. S.; Matarazzo, S. V.; 2005. Teor de ácido linoléico conjugado na gordura do leite de búfalos. *Revista do Instituto de Laticínios "Cândido Tostes"*, 60:346/347, 79-86. Fievez V.; Vlaeminck, B.; Dhanoa, M. S. 2003. Use of principal components analysis to investigat the origin of heptadecenoic acid and conjugated linoleic acids in milk. *Journal of Dairy Science*, 86,1017-1053. Hara, A.; Radim, N. S. 1998. Lipid extraction of tissues with low toxicity solvent. *Analytical Biochemistry*, 90, 420-426. Kay, J. K.; Macle, T. R.; Auldist, M. J. 2004. Endogenous synthesis of *cis-9, trans-11* conjugated linoleic acid in dairy cows fed fresh pasture. *Journal of Dairy Science*, 87, 369-378. Lock, A. L.; Garnsworthy, P. C. 2003. Seasonal variation in milk conjugated linoleic acid and Δ^9 -desaturase activity in dairy cows. *Livestock Production Science*, 79, 47-59.