

Leptin in milk and plasma of dairy asses

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ABSTRACT - Milk and plasma leptin levels have been studied in dairy asses machine milked according to two different routines: 20 pregnant, pluriparous asses, were divided into two groups subjected, every 28 d for 150 d, to two consecutive milkings carried out at different intervals, i.e. 20 vs. 4 hours interval, respectively for group A and group B. During the study, the declining total milk obtained by machine milking was unaffected by the different milking strategies; body condition score of asses as well did not vary between the groups. Different milking intervals did not significantly influence skimmed milk leptin content neither plasma leptin level. Moreover, we did not find significant variation in plasma leptin neither correlation with BCS, indicating that in donkey pregnancy inhibits the cross talk between hypothalamus and adipose tissue.

Key words: Dairy asses, Leptin, Plasma and milk.

INTRODUCTION - Besides its compositional resemblance to human milk, ass's milk is characterized by a high variability of its constituents; moreover, literature data show that not only milking can influence milk yield and composition of *Equidae* (Doreau, 1991; Salimei and Chiofalo, 2006), but also the management of machine milking can affect essential aspects of ass's milk, such as fat content, hygiene and healthy status of udder (Salimei *et al.*, 2006). Donkey milk, on the other hand, has been regarded as a promising hypoallergenic food for infants, even though it needs to be appropriately adapted in chemical composition and carefully evaluated from the hygienic point of view (Restani *et al.*, 2002).

Among the functional components of milk, it is interesting to note that leptin, a metabolism modifier that plays a crucial role in coordinating food intake, energy expenditure and nutrient utilization (Houseknecht *et al.*, 1998), shows levels in ass's milk comparable to values observed also in human milk (Salimei *et al.*, 2005), adding value to its characterization. In not pregnant dairy donkeys, milk leptin did not show significant variation during lactation, while leptinemia was found significantly related to both days in milking and body condition score (Maglieri *et al.*, 2006).

Because in many species plasma leptin has been demonstrated an important endocrine signal of nutritional status and body fatness involved in the response to physiological and environmental stimuli (Chilliard *et al.*, 2005), in order to contribute to the knowledge on physiology of donkeys involved in an innovative food chain for infancy, our objective was to investigate on milk and plasma leptin levels in pregnant dairy asses during mid-late lactation, as also related to different machine milking routines.

MATERIAL AND METHODS - Twenty pluriparous asses (89 ± 42 days of lactation), pregnant at the beginning of the trial, and machine milked, were divided into two homogeneous groups (A: 382.5 ± 86kg BW; B: 342.8 ± 55kg BW) and were subjected to two consecutive milkings with different intervals within 24 hours: group A was milked at 15:00h and 11:00h of the subsequent day (20 hours interval between the two milkings) and group B was milked at 11:00h and 15:00h (4 hours interval). Asses were stabled with their foals in two boxes provided with a wide external paddock; foals were physically separated from the dams since 3 hours before each milking. The animals were daily fed in average 8kg meadow hay and 2.5kg of mixed feed, divided in two meals. The study lasted 150 days; every 28 days individual milk yield was recorded and bulk milk samples from each group were centrifuged (1500xg, 20 min, 4 °C) and stored at - 20°C until leptin analysis.

With the same schedule, donkeys' body condition was monitored on a 0 to 5 scale, and blood samples were taken, after the morning milking and before feeding.

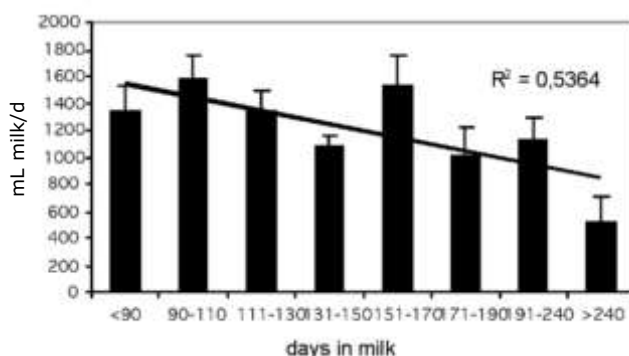
Both skimmed milk and plasma leptin levels have been assayed with multi-species leptin RIA kit (Linco Research Inc, St. Charles, MO), already validated for equine plasma. Intra-assay and inter-assay coefficients of variation were 3.8% and 7.8%, respectively.

Data were analyzed by analysis of variance, considering the covariate effect of days of lactation; correlations between the investigated parameters were also tested (SAS Inc. Cary, NC, USA).

RESULTS AND CONCLUSIONS - Donkeys showed a rapid adaptation to the experimental conditions and their body condition score did not differ between the two milking strategies (A: 3.50 ± 0.04 ; B: 3.49 ± 0.04); body condition of asses did not vary significantly either during lactation, in contrast to what observed in non pregnant dairy asses (Maglieri *et al.*, 2006).

Total milk obtained by machine milking in 24 hours was unaffected by milking strategy (A: 1365.77 ± 91.7 mL-d-1; B: 1096.68 ± 92.77 mL-d-1); besides, a significant decline of total milk extracted has been observed during lactation (figure 1), consistently to literature data (Salimei and Chiofalo, 2006).

Figure 1. Daily ass's milk yield.



Leptin, the milkborne growth factor, in skimmed bulk milk resulted unaffected by the milking routine (A: 4.78 ± 0.21 ng leptin-mL⁻¹; B: 5.32 ± 0.21 ng leptin-mL⁻¹). The observed leptin values are consistent with data reported in literature for many mammals (Bonnet *et al.*, 2005); besides, the levels of leptin found in milk from pregnant dairy asses are, on average, higher than those measured in individual skimmed milk of non pregnant dairy asses (Salimei *et al.*, 2005).

Dairy donkey's leptinemia did not differ significantly between the two experimental groups (A: 5.63 ± 0.38 ng leptin-mL⁻¹; B: 5.70 ± 0.38 ng leptin-mL⁻¹). The plasma levels of leptin here reported were lower than those observed in lactating, non pregnant dairy donkeys (Maglieri *et al.*, 2006) and did not vary during lactation (Figure 2). Moreover, plasma leptin levels and body condition score were not related, in contrast to what observed in non pregnant dairy asses (Maglieri *et al.*, 2006), but in accordance to Gentry *et al.* (2002) finding on mares with high BCS.

Lactation in equids reduces leptinemia and also pregnancy can reduce the plasma leptin level to stimulate food intake and to maintain BCS in a period of elevated energy requirement (Heidler *et al.*, 2003). However, the absence of a relation between plasma leptin and body condition score in

our results indicates that the interaction of pregnancy with lactation may alter the hypothalamus-adipose tissue axis in donkeys, in order to maintain the body energy stores. More in depth studies could therefore clarify the role of variations in leptinemia as related to the physiological status of donkey and, possibly, how intensive husbandry strategies might interact with the adaptive capacities of this docile specie in the innovative farming environment.

Figure 2. Leptinemia during lactation.

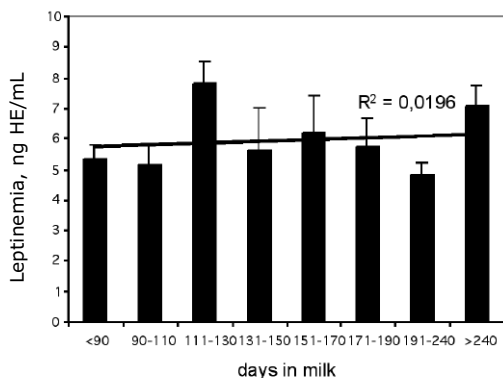
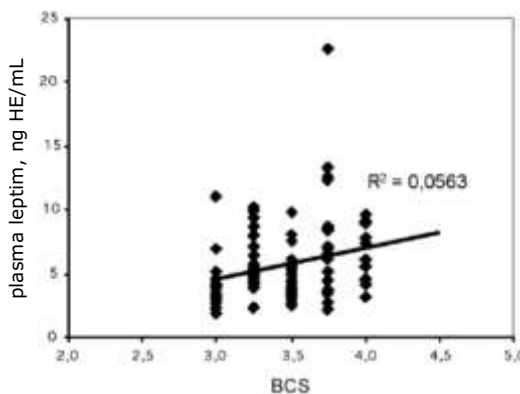


Figure 3. Leptinemia and BCS.



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REFERENCES - Bonnet M., Delavaud C., Rouel J., Chilliard Y., 2005. Pregnancy increases plasma leptin in nulliparous but not primiparous goats while lactation depresses it. *Domest. Anim. Endocrinol.* 28: 216-223. Chilliard Y., Delavaud C., Bonnet M., 2005. Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. *Domest. Anim. Endocrinol.* 29 (suppl.1): 3-22. Doreau M., 1991. Le lait de jument. *INRA Prod. Anim.* 4: 297-302. Gentry LR, Thompson Jr DL, Gentry Jr GT, Davis KA, Godke RA, Cartmill JA., 2002. The relationship between body condition, leptin, and reproductive and hormonal characteristics of mares during the seasonal anovulatory period. *J Anim Sci.* 80:2695-703. Heidler B., Parvizi N., Sauerwein H., Bruckmaier R.M., 2003. Effects of lactation on metabolic and reproductive hormones in Lipizzaner mares. *Dom. Anim. Endocrinol.* 25: 47-59. Houseknecht K.L., C.A. Baile, R.L. Matteri, M.E. Spurlock, 1998. The biology of leptin: a review. *J. Anim. Sci.* 76: 1405-1420. Maglieri C., Rosi F., Magistrelli D., Polidori M., Fantuz F., Salimei E., 2006. Different fibre sources in dairy ass's diet: effects on body condition and plasma leptin level. *EAAP publ.* 120, Wageningen Academy Press, Wageningen, NL: 167-168. Restani P., Beretta B., Flocchi A., Ballabio C., Galli CL., 2002. Cross reactivity between mammalian proteins. *Ann. Allergy Asthma Immunol.*, 89: 11-15. Salimei E., Chiofalo B., 2006. Asses: milk yield and composition. *EAAP publ.* 120, Wageningen Academy Press, Wageningen, NL: 117-132. Salimei E., Maglieri C., Polidori M., Succi R., Chiofalo B., Gambacorta M., Varisco G., 2006. Machine milking of dairy asses as related to milk production and body condition. *EAAP publ.* 120, Wageningen Academy Press, Wageningen, NL: 181-182. Salimei E., Rosi F., Maglieri C., Polidori M., Fantuz F., Varisco G., 2005. Effetti di differenti fonti fibrose nella dieta di asine sul contenuto di leptina e dei principali costituenti del latte. *Proc. 7th SIDI Congress, Lodi (LO, Italy)* 22-23 July: 160-165.