

# Bioactive components in goat milk and plasma

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## ABSTRACT

Leptin, ghrelin and IGF-1 were measured in sonicated defatted milk (morning and evening milkings) and in plasma (before feeding, 1 and 4 h after feeding) in 17 Saanen goats in mid lactation. Leptin and ghrelin were positively correlated and resulted twice/thrice more concentrated in milk than in plasma. Plasma IGF-1 level was 7-fold higher than in milk with high correlation ( $r=+0.84$ ;  $P<0.01$ ). During periprandial period (immediately pre-feeding, 1 and 4-h post feeding) plasma leptin and IGF-1 levels did not change significantly, while plasma ghrelin level was significantly lower 1 h after feeding.

KEY WORDS: ghrelin, IGF-1, leptin, dairy goat, periprandial variation, milk

## INTRODUCTION

Mammalian milk contains enzymes, nutrients, hormones and protective ad trophic factors; some of them diffuse from plasma, others are secreted and others are encrypted into milk protein. Leptin and IGF-1 are growth factors identified in plasma and in milk; in the latter they are implicated in neonatal maturation, since they may be involved in the regulation of growth, in the development and maturation of the neonatal gut, and of the immune and neuroendocrine system (Blum and Baumrucker, 2002; Wolinski et al., 2003). Recent studies suggest that also ghrelin may be involved in neonatal development (Hayashida et al., 2002).

Due to the importance of goat milk in newborn and human feeding, the aim of this study was to evaluate the presence of leptin, ghrelin and IGF-1 in goat milk. To contribute to the study of the origin of these milk-borne components, we investigated also their relationships with plasma levels. Moreover, since these peptides are involved in the control of satiety, another aim was to determine their plasma variation during the periprandial period.

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## MATERIAL AND METHODS

Seventeen Saanen goats in mid lactation were fed a diet based on mixed hay, maize, beet pulp, barley and soyabean meal (40:60 forage to concentrate ratio) at 9.00 and 17.00 h, and milked at 8.00 and at 18.00 h.

At 133, 148 and 163 DIM (days in milk), milk production was recorded at each milking and individual samples were collected and stored at -20°C until analysis. Milk samples, sonicated on ice for 1 min with an ultrasonic homogenizer and centrifuged (2.150 xg for 30 min at 4°C), were tested for milk leptin (multi-species leptin RIA, Linco Res. Inc., St. Charles, MO, USA), IGF-1 (IGF-1 RIA with extraction, Diagnostic System Laboratories, Inc., Webster, TX, USA) and ghrelin (total active Ghrelin RIA, Linco Res. Inc., St. Charles, MO, USA) content.

At the same DIM, five ml of jugular vein blood were taken before the first feeding of the day, 1 and 4 h after the feeding. The samples were collected into EDTA tubes, centrifuged (6.000 xg for 15 min at 10°C) and plasma stored at -20°C, until analysis for leptin, IGF-1 and ghrelin by the same RIA method employed for milk. These methods, using antibody directed against human leptin, ghrelin and IGF-1, were validated for goat plasma and milk verifying the parallelism to the standard curve, of serial dilutions (25 to 100 µl) of plasma and milk in 100 µl buffer.

Milk production, milk and blood variables were analysed using the GLM procedure of SAS (1989).

## RESULTS

Milk production was 30% higher at morning and milk IGF-1 level was thrice higher in morning than in evening milking. Milk leptin and ghrelin levels did not show significant differences between morning and evening milkings (Table 1).

Table 1. Levels of hormones in milk at morning and afternoon milkings

	Morning milking	Evening milking	SE	P
Milk, g/milking	1317	1026	62	<0.01
Leptin, ng/ml	7.81	7.64	0.83	NS
Ghrelin, ng/ml	3.41	3.40	0.09	NS
IGF-1, ng/ml	13.11	4.71	1.6	<0.01

During periprandial period (immediately pre-feeding, 1 and 4-h post feeding) plasma leptin and IGF-1 levels did not differ significantly, while plasma ghrelin level was lower 1-h post feeding than before feeding (Table 2).

Both in plasma and in milk, leptin levels were positively correlated to ghrelin and negatively to IGF-1 levels. The correlation between ghrelin and IGF-1 was significant only in plasma. Milk and plasma leptin were not correlated, but milk ghrelin and milk IGF-1 were significantly correlated to their plasma levels (Table 3):

Table 2. Levels of hormone in plasma during the periprandial period

Hormone, ng/ml	Before feeding	1-h post feeding	4-h post feeding	SE	P
Leptin	2.80	2.61	2.58	0.11	ns
Ghrelin	1.34 <sup>a</sup>	1.23 <sup>b</sup>	1.27 <sup>a</sup>	0.03	<0.05
IGF-1	65.2	74.6	77.3	14.1	ns

<sup>a,b</sup> values in a row without the same suffix differ significantly

Table 3. Correlation between variables in milk and plasma

	R	n	P
Leptin/ghrelin			
plasma	+0.43	51	<0.01
milk	+0.38	34	<0.05
<i>Leptin/IGF-1</i>			
plasma	-0.43	51	<0.01
milk	-0.47	34	<0.05
Ghrelin/IGF-1			
plasma	-0.41	51	<0.01
milk	-0.15	34	NS
<i>Milk/plasma</i>			
leptin	+0.23	34	NS
ghrelin	+0.47	„	<0.01
igf-1	+0.84	„	<0.01

As reported in Figure 1, plasma leptin and ghrelin levels were 2-3 folds lower than milk levels; on the contrary plasma IGF-1 was 7 folds higher than milk IGF-1.

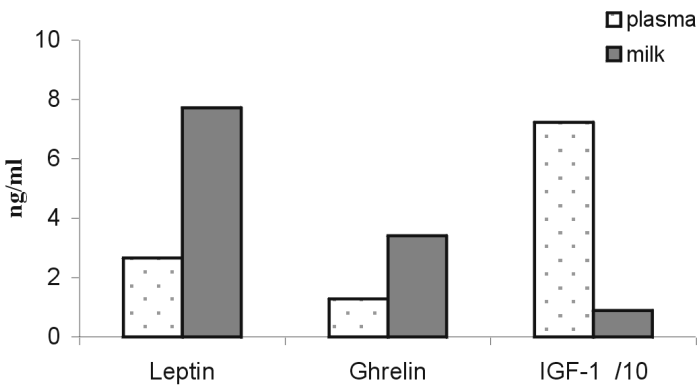


Figure 1. Hormones in plasma and milk. For each variable, plasma and milk levels are significantly different (P<0.01)

## DISCUSSION

Milk production was significantly higher at morning than at evening, indicating a constant milk synthesis rate through the day.

In literature the results regarding periprandial variation of leptin in ruminants are conflicting; in fact post-prandial increments (Marie et al., 2001), decrements (Delavaud et al., 2002) or no variation are reported (Daniel et al., 2002). In this study no variation is observed in plasma leptin, indicating that the buffering effect of the rumen can modulate the absorption of nutrients and dim the post-prandial changes in plasma leptin, detected in monogastric species.

In sheep, plasma ghrelin is reported to increase just before feeding, and to be involved in inducing GH surge during feeding (Sugino et al., 2003). Analogously, in goat we observed the same periprandial variation, although lower. However, to explain the periprandial variation of ghrelin, further investigation should be required.

The milk levels of leptin and ghrelin were twice/thrice higher than their plasma levels, suggesting mammary synthesis or concentration. Although one site of action of milk-borne growth factors may be at intestinal level, the transfer of leptin from milk to the neonate blood has been shown for leptin in rat (Casabiell et al., 1997) and for IGF-1 from indirect evidence in calf (Sparks et al., 2003).

Further research is needed to determine whether intraspecific or interspecific transfer can occur and whether these components from goat milk can maintain their biological activity in the systemic circulation of the neonate.

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