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Effect of duodenal ornithine infusion in cows on milk production and blood plasma constituents at two levels of feeding

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Introduction

Postruminal infusion of casein in ruminants generally increases milk production and, particularly, mammary protein secretion (CLARK 1975). Several authors attempted to reproduce these effects by postruminal infusion of a wide range of casein amino acids, whether individually or mixed, and obtained variable results (SCHWAB et al. 1976). The effect of postruminal infusion of ornithine has not been studied.

Although not present in milk protein, an appreciable uptake of ornithine from the blood by the mammary gland was found by VERBEKE and PEETERS (1965). Its hydrocarbon skeleton acts primarily as a precursor for the synthesis of proline in the udder (VERBEKE et al. 1968), whereas its δ-amino group contributes nitrogen for the synthesis of numerous nonessential amino acids (ROETS et al. 1979). Ornithine also can be converted into polyamines as spermidine and spermine (SCHEPARTZ 1973), which are required for the synthesis of milk protein in mammary gland cultures (OKA and PERRY 1974; RILLEMA 1976). Intravenous infusion of ornithine released growth hormone (GH) and prolactin in sheep (HANDWERGER et al. 1981).

In the present work, we have studied the effect of duodenal ornithine infusion on milk secretion and levels of several plasma constituents of cows fed with diets providing either 110% or 75% of total energy and protein requirements.

Experimental procedure

Animals. Four experiments were conducted with two lactating cows of the Friesian breed (no. 103 and 104) fitted with a T-cannula in the proximal duodenum. The surgical technique was described previously by GODEAU and D'IETEREN (1976). The animals were 3 and 5 yr old and weighed 550 and 500 kg, respectively, at beginning of the experiments.

Design and Treatments. In Exp. 1, the two cows had calved since 6 wk when they were fed on a diet consisting of 110% of the energy and protein requirements according to the Dutch feeding standards used in our country (BUYSSE et al. 1977). After an adaptation period of at

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least 2 wk, an ornithine solution was continuously infused into the duodenum of cow 103, while an equivalent amount of a blank solution was infused into the duodenum of cow 104. From d 3 to 9 of infusion, milk yield was recorded and analyzed. During the last day, blood samples were taken at 09.00, 11.00, 13.00, 15.00 and 17.00 h via an indwelling catheter that was inserted into an external jugular vein the day before sampling. Thereafter, the treatments were reversed, i.e., cow 103 received the blank and cow 104 the ornithine solution.

In Exp. 2, immediately after the first experiment, the cows were fed on a diet consisting of 75% of the energy and protein requirements. Adaptation period and experimental design were identical to the previous.

One year later when the animals had calved again, these experiments were repeated except that the order of the diets was reversed, and that cow 103 received first the blank solution and cow 104 the ornithine solution (Exp. 3 and 4). At beginning of these trials, the cows were in the third month of lactation. Only four amino acids were assayed in blood plasma (ornithine, lysine, histidine, and arginine). The sequences of treatments are given in table 1.

Diets. Ingredients of the diets and dry matter intake are shown in table 2. They were fed in two equal parts at 06.30 and 16.00 h. Water was available ad libitum.

Table 1

Sequences of treatments

Experiment	Year	Level of intake (% of requirements)	Cow 103	Cow 104
I	1	110	Ornithine Blank	Blank Ornithine
II	1	75	Ornithine Blank	Blank Ornithine
III	2	75	Blank Ornithine	Ornithine Blank
IV	2	110	Blank Ornithine	Ornithine Blank

Table 2

Composition and intake of experimental diets

Item	Experimental diet number			
	1 HI[1]	2 LI[2]	3 LI	4 HI
Ingredients, % DMB[3]				
Meadow hay	50.4	52.8	43.9	37.1
Maize silage	16.4	17.3		
Dehydrated maize			32.3	32.7
Soybean meal			8.7	7.3
Commercial concentrates	32.6	28.5	12.6	21.3
Mineral mixture	.6	1.4	2.5	1.6
Component				
Dry matter, kg/d	14.6	7.0	8.4	12.4

[1] High energy and protein intake (110% of requirements).
[2] Low energy and protein intake (75% of requirements).
[3] Percent, dry matter basis.

Ornithine Infusion. Every day, 51.2 g of L-(+)-ornithine-hydrochloride[5] equivalent to 40 g of ornithine, were dissolved in 2 liters of previously boiled water, and acidified to pH 3.

The solution was continuously infused into the duodenum of one animal using a peristaltic pump[6]. The infusion rate corresponded to a daily administration of 36.1 g of ornithine. The other cow received the same amount of fluid without ornithine (blank).

Analytical Procedures. Milk yield was recorded daily, and analyzed for milkfat according to the method described by LING (1949) and for crude protein by the Kjeldahl procedure. Lactose was determined according to CONETTA et al. (1970); milk energy was measured as described by DE BAERE et al. (1966). Blood samples were collected in heparinized tubes containing 4 mg NaF. Plasma was separated by centrifugation (3,000 rpm during 40 min) and stored at −20°C until analyses. Methods used for determination of plasma amino acids and glucose have been described (PEETERS et al. 1979). Plasma protein was measured by the biuret technique[7]. Radio-imunoassays were used to measure plasma concentrations of GH (REYNAERT and FRANCHIMONT 1974) and insulin (MICHAUX et al. 1981). Sensivity of GH[8] and insulin[9] determinations was .5 ng/ml and 1.3 µUI/ml, respectively; inter- and intra-assay coefficients of variance were 2.0 and 1.5% for GH and 11.8 and 17.0% for insulin, respectively.

Between treatments (ornithine or blank), milk composition and milk yield were compared according to a 2 × 2 × 6 arrangement (2 treatments, 2 cows, 6 d); for the blood constituents, a 2 × 2 × 5 arrangement was used (2 treatments, 2 cows, 5 blood samplings). As year had no influence on response to treatments, it was omitted from the model. Analysis of variance was performed using GLM − SAS (SAS 1979).

Results

Concentration of milk constituents and daily milk yields are shown in table 3. Duodenal infusion of ornithine produced insignificant variations of milk composition and milk yields when high energy and high protein diets were fed. On the contrary significant increases were recorded for lactose concentration, milk production, 4%-fat corrected milk yield, and daily yields of fat and lactose after infusion of ornithine in underfed animals.

Mean concentrations of free amino acids in blood plasma are presented in table 4. They were not significantly affected by time of sampling. Ornithine infusion in the overfed animals significantly increased ornithine levels, proline, arginine, alanine and lysine. Ornithine infusion augmented 16 of 18 amino acids in cows on low energy and low protein diets. This increase was significant for ornithine, citrulline, proline, serine, glutamine, aspartic acid, lysine, arginine, valine, phenylalanine, total essential and nonessential amino acids. In contrast, histidine and methionine decreased.

The mean concentrations of glucose, protein, GH and insulin in blood plasma are presented in table 5. The administration of ornithine significantly increased the glucose and protein levels in the underfed cows. Ornithine further enhanced GH concentration with low energy and low protein diets, but not in overfed animals. Ornithine administration did not affect insulin.

[5] Janssen Chimica, Belgium.
[6] Pump I, Technicon, Belgium.
[7] Gilford Diagnostics, Cleveland.
[8] Pentex Biochemicals, Miles Laboratories, Naperville (1 UFP/mg).
[9] b insulin, Sigma, St. Louis (24.5 UI/mg).

Table 3

Effect of duodenal ornithine infusion on milk composition and milk yield

Item	High intake: Blank	High intake: Ornithine	Low intake: Blank	Low intake: Ornithine	SE
Composition of milk					
Fat, g/liter	38.8	39.4	38.1	39.5	1.2
Protein, g/liter	28.9	29.2	25.4	24,6	.4
Lactose, g/liter	49.4	48.4	44.9[a]	47.3[a]	.8
Energy, MJ/liter	3.0	3.0	2.8	2.9	.1
Production					
Milk yield, liter/d	16.0	15.7	11.1[a]	11.7[a]	.5
4%-fat-corrected milk, liter/d	15.6	15.5	10.7[a]	11.5[a]	.5
Fat, g/d	611	612	417[a]	453[a]	20.7
Protein, g/d	459	456	287	293	15.3
Lactose, g/d	780	758	497[a]	544[a]	24.9
Energy, MJ/d	47.8	48.2	31.6	33.2	2.3

[a] Results in same line with common superscripts are different ($P < .05$)

Table 4

Effect of duodenal ornithine infusion on amino acid level in blood plasma

(µmoles/100 ml)

Amino acids	High intake: Blank	High intake: Ornithine	Low intake: Blank	Low intake: Ornithine	SE
Lysine (20)[1]	5.3[de]	6.7[df]	6.8[be]	7.9[bf]	.41
Histidine (20)	4.9	4.3	5.4[d]	4.8[d]	.19
Arginine (20)	5.3[bh]	6.1[bi]	6.6[gh]	8.2[gi]	.40
Threonine (10)	7.0[b]	6.8[c]	4.6[b]	5.6[c]	.76
Valine (10)	16.9	17.9	14.5[d]	20.1[d]	1.62
Methionine (10)	1.6	1.5	1.7	1.4	.20
Isoleucine (10)	8.0	8.5	6.2	7.6	.76
Leucine (10)	9.2	9.8	8.5	8.5	.84
Tyrosine (10)	3.7[d]	3.5[e]	2.8[d]	3.0[e]	.17
Phenylalanine (10)	3.9	3.6	3.3[b]	3.8[b]	.18
Total EAA (10)	65.8	68.7	60.4[d]	70.9[d]	4.44
Ornithine (20)	4.4[g]	7.7[gi]	4.8[h]	11.2[hi]	.38
Citrulline (10)	6.3	5.5[h]	5.8[g]	7.6[gh]	.40
Proline (10)	6.5[d]	8.1[bd]	6.2[e]	9.3[be]	.70
Serine (10)	7.6	7.1	6.3[b]	7.3[b]	.38
Glutamine (10)	21.5	18.1[d]	20.7[b]	24.5[bd]	1.10
Glycine (10)	24.7	25.9[d]	26.9	30.0[b]	1.47
Alanine (10)	15.5[b]	18.4[b]	17.4	19.2	2.25
Aspartic acid (10)	1.7	1.7	1.4[b]	1.7[b]	.15
Total non EAA (10)	88.1	92.4[h]	89.3[g]	110.7[gh]	4.53

[1] The number of samples analyzed per treatment and per diet is in parentheses.
Results in same line with common superscripts are different: b, c ($P < .05$); d, e, f ($P < .01$); g, h, i ($P < .001$).

Table 5

Effect of infusion of ornithine into the duodenum on glucose, protein, bGH and insulin levels in blood plasma

Constituents[1]	High intake		Low intake		SE
	Blank	Ornithine	Blank	Ornithine	
Glucose, mg/100 ml	58.3	60.2	53.3[c]	61.6[c]	1.05
Protein, g/100 ml	11.0	10.8[d]	10.8[c]	12.0[cd]	.40
bGH, ng/ml	10.0[d]	8.1[e]	15.1[bd]	20.1[be]	1.27
Insulin, µUI/ml	43.3[e]	39.6[f]	23.2[e]	25.2[f]	2.71

[1] Twenty samples were analyzed per treatment and per diet for each constituent.
Results in same line with common superscripts are different: b ($P < .05$); c, d ($P < .01$); e, f ($P < .001$).

Discussion

Our results agree with those of Handwerger et al. (1981) who stated that ornithine is a potent stimulus to the secretion of GH in sheep. Ornithine infusion increased GH levels only when low energy and low protein diets were given, while no significant effect appeared during overfeeding. There is evidence that fasting alters sensitivity of the pituitary to factors that cause secretion of GH, such as arginine (Trenkle 1978) and thyrotropin releasing hormone (Bauman et al. 1979). Concentration of insulin was low in the blood of animals suffering from an energy deficit. This agrees with previous results (Trenkle 1978).

Duodenal infusion of ornithine augmented ornithine levels of the plasma. Reactions of Henseleit Krebs cycle in the liver and metabolic reactions in the gut wall and kidney (Bergman et al. 1974) are involved in the increase of arginine and citrulline in the plasma. Deamination of ornithine catalysed by ornithine-δ-transaminase causes an increase of proline and may be involved to some extent in increased plasma levels of glucose, aspartic acid, glutamine, glycine, alanine and serine (Schepartz 1973). It can be deduced that ornithine, by its metabolic reactions in mammary gland and other tissues, is supplying precursors of milk constituents and may have a stimulating effect on milk synthesis in cows suffering from an energy and protein deficit.

Infusion of ornithine had no noticeable effect on milk production when cows were fed at 110% of feeding standards. However, the productions of milk, fat, lactose and milk energy were substantially increased by ornithine when diets supplied only 75% of requirements. This increase was accompanied with a higher GH content in plasma. As bGH administration has consistently resulted in marked increases of milk production and yield of fat, protein, lactose and milk energy (Machlin 1973; Bines et al. 1980; Peel et al. 1981), it is likely that release of GH was involved, at least partly, in the increased milk secretion after administration of ornithine in cows suffering from an energy and protein deficit. The beneficial effect of GH on milk secretion likely was enhanced by low plasma levels of insulin in these animals. Insulin is associated with many processes that direct energy away from milk synthesis and towards body tissues. It has been hypothesized that ratio of GH to insulin in plasma may be a key regulator in the partitioning of absorbed nutrients for milk production in cows (Bines et al. 1980).

A marked increase in glucose supply in the underfed animals must have occurred after ornithine infusion, since plasma glucose rose significantly and lactose production increased from 497 to 544 g/d ($P < .05$). Clearly this increase was not induced solely by gluconeogenesis from ornithine administered at a dose of 36.1 g/d. BINES and HART (1982) suggested that GH may increase glucose supply to the mammary gland by decreasing utilization of glucose elsewhere rather than by a general increase in glucose production.

Ornithine had little stimulating effect on milk protein secretion in the underfed cows. This agrees with the results of PEEL et al. (1981) who found that milk protein secretion was unchanged after administration of bGH in cows receiving a low energy and low protein diet. Ornithine increased the concentration of 16 individual amino acids but decreased methionine and histidine levels in jugular plasma. This may indicate that entry rates of most amino acids were in excess of requirements for milkprotein snythesis, but that methionine and histidine were limiting.

Our experiments have shown that infusion of the non-casein amino acid ornithine into the duodenum increases milk secretion in underfed cows, and that GH likely is involved in this phenomen.

Summary

An ornithine solution, corresponding to 36.1 g ornithine/d, or an equivalent blank solution were each continuously infused into the duodenum of two lactating cows of the Friesian breed. The cows were fed either on a high energy and high protein diet (110% of energy and protein requirements) or on a low energy and low protein diet (75% of requirements). Milk composition and milk yield were recorded, and blood plasma contents of amino acids, glucose, growth hormone (GH) and insulin were analysed. The procedure was repeated 1 year later.

Ornithine had no effect on milk yields or blood components when the high energy and high protein diets were given. On the contrary, it significantly increased milk, lactose, and fat production with the low energy and low protein diets, while milk protein secretion was only slightly affected. The levels of nonessential and essential amino acids in blood plasma were enhanced, except for histidine and methionine. The latter amino acids likely were limiting for milk protein synthesis.

An increase in glucose supply in the underfed animals likely occurred after ornithine administration, since there was a significant increase of plasma glucose and protein levels and total lactose production.

Ornithine infusion increased the GH secretion only when the low energy and low protein diets were given.

It was concluded that infusion of the non-casein amino acid ornithine into the duodenum increases milk secretion in underfed cows, and that GH likely is involved in this phenomen.

Zusammenfassung

Effekt einer Ornithininfusion im Dünndarm von Kühen auf die Milchproduktion und einiger Blutplasmaparameter bei unterschiedlicher Fütterungsintensität

Täglich wurde eine Lösung mit 36,1 g Ornithin oder die entsprechende Null-Lösung kontinuierlich in den Dünndarm von zwei Milchkühen verabreicht. Die Rationen entsprachen 110% oder 75% des Energie- und Eiweißbedarfs. Die Milchproduktion und Milchzusammensetzung wurden gemessen und der Blutplasmagehalt an Aminosäuren, Glukose, Wachstumhormon (GH) und Insulin analysiert. Das Experiment wurde ein Jahr später wiederholt.

Die Ornithinzugabe hatte keinen Einfluß auf die Milchproduktion und die Blutplasmazusammensetzung bei intensiver gefütterten Tieren. Dagegen wurden signifikant höhere Milch-,

Laktose- und Fettgehalte gemessen bei nicht so intensiv gefütterten Tieren, während die Eiweißproduktion nicht beeinflußt wurde. Der Blutplasmagehalt an essentiellen und nicht essentiellen Aminosäuren stieg an, außer bei Histidin und Methionin. Diese beiden Aminosäuren können als limitierend für die Milcheiweißsynthese angesehen werden.

Der Glukoseumsatz wurde ebenfalls durch die Ornithinzufuhr bei Unterfütterung verändert, da der Glukose- und Eiweißspiegel im Blutplasma und die Laktoseproduktion signifikant anstiegen.

Die Ornithinzufuhr erhöhte ebenfalls die GH-Sekretion bei Unterfütterung.

Die vorliegenden Ergebnisse haben gezeigt, daß eine Ornithininfusion in den Dünndarm von Kühen die Milchproduktion stimuliert, wenn die Energie- und Eiweißversorgung unterhalb des Bedarfes liegt. Diese Feststellung wird durch die erhöhte GH-Sekretion bestätigt.

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