

# Responses of mammary amino acid metabolism and aminopeptidase N gene expression to duodenal soyabean small peptides and infusion of free amino acids in lactating goats

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(Received 24 February 2009; revised version 7 October 2009; accepted 29 January 2010)

## ABSTRACT

The duodenal perfusion technique was used to determine whether small peptides were more effective than free amino acids (FAA) for milk protein synthesis and to investigate the effects of infusing soyabean small peptides (SSP) or their FAA on the mammary amino acid (AA) metabolism of lactating goats. Six Saanen goats with silicon catheters implanted into their carotid artery and mammary veins were used in a Latin square design. Blood samples were collected from the carotid artery and mammary vein. The AA concentrations in the plasma, as well as mammary AA uptakes were monitored. The results showed that the concentrations of most essential amino acids (EAA) were significantly higher ( $P < 0.05$ ) in the FAA infusion treatment as compared with the SSP treatment, except for Val, Ile, and Met. The concentration of most of the non-essential amino acids (NEAA), measured after FAA infusion, was highest ( $P < 0.05$ ) when compared with the SSP treatment and the controls. The mammary uptake of most of EAA in the FAA infusion group was higher in comparison with the SSP infusion group. The mammary uptake to milk output ratios of EAA (except for Met) were low in the SSP treatment, compared with the FAA treatment. Significant increases in milk protein yield and milk protein content in the FAA infusion treatment were observed ( $P < 0.05$ ). In the SSP infusion treatment, milk yield and

\* Supported by the National Natural Science Foundation of China (Grant no. 30671521)

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milk protein were numerically increased compared with the control. The abundance of aminopeptidase N (APN) mRNA in mammary tissue was 38.28 in the SSP infusion group, and 2.83, 5.28 in the control and FAA infusion treatment, respectively. In conclusion, the data suggest the FAA was more effective for milk protein synthesis, while the activity of APN was related to utilization of small peptides by the mammary gland.

KEY WORDS: lactating goats, mammary metabolism, milk protein, soyabean small peptides, free amino acids

## INTRODUCTION

Numerous studies report that free amino acids (FAA) and peptide-bound amino acids (PBAA) are the sources for milk protein synthesized by the mammary gland (Tagari et al., 2004, 2008; Mabjeesh et al., 2005). Some of the amino acids (AA) circulating in blood plasma are in the form of peptides (Seal and Parker, 1991; Koeln et al., 1993), and variable contributions of PBAA to the total concentration of AA have been reported (Remond et al., 2000, Tagari et al., 2004). When FAA from blood plasma cannot meet the demand for milk protein synthesis, the deficit is supplied by PBAA. *In vivo* and *in vitro* studies have proven that PBAA can be utilized for protein synthesis by glandular tissue (Emmerson and Phang, 1993; Pan et al., 1996). The absorption mechanisms of PBAA differ, however, from those of FAA. PBAA can be absorbed as intact peptides, *via* peptide transporters, and the absorption of PBAA in the small intestine is fast (Addison and Matthews, 1972). Although many studies have shown that small peptides can be used effectively for protein synthesis by the mammary gland *in vitro* experiments, methionine-containing peptides were observed to elicit greater protein accretion than free methionine in MAC-T, when the cell medium contained the same concentrations of free methionine and methionine-containing dipeptides (Pan et al., 1996). The metabolisms of small peptides and FAA are complex processes *in vivo*, however, and it is still unclear whether small peptides are more effective than FAA for protein synthesis. As the mRNA of peptide transporters, PepT1, is not detected concomitantly in the mammary glands of cows (Chen et al., 1999), it is still unclear how PBAA are used by this gland. It was shown recently that aminopeptidase N (APN) was expressed in the mammary tissue of goats and cows (Mabjeesh et al., 2001). Therefore, it might be involved in the utilization and absorption of small peptides by the mammary gland.

The purpose of this experiment was to investigate the effect of duodenal infusion of FAA and PBAA on milk protein synthesis and expression of APN mRNA in the mammary gland.

## MATERIAL AND METHODS

*Material*

The SSP composed of dipeptides and tripeptides was purchased from ZS Duqing Biotech CO., Ltd. (Shandong, China). The AA composition in the SSP is shown in Table 1. According to the producer's declaration, free AA made up <60 g/kg of SSP and oligopeptides, <30 g/kg. Note that the PBAA content of SSP was calculated as the difference between the AA content of hydrolysed samples and the FAA content of the same sample before hydrolysis. The FAA were bought from Bio Basic Inc. (Markham Ontario, Canada).

Table 1. Total peptide-bound amino acids content of soyabean small peptides (SSP, n=4)

Amino acids	Concentrations, mg/g
Valine (Val)	27.29 ± 3.02
Isoleucine (Ile)	30.60 ± 3.65
Leucine (Leu)	38.50 ± 3.92
Threonine (Thr)	20.28 ± 2.17
Methionine (Met)	8.37 ± 0.62
Phenylalanine (Phe)	23.76 ± 2.31
Lysine (Lys)	33.55 ± 3.62
Histidine (His)	15.71 ± 1.55
Arginine (Arg)	44.70 ± 4.29
Serine (Ser)	24.31 ± 2.63
Glutamic acid/glutamate (Glu/Gln)	101.76 ± 11.58
Glycine (Gly)	27.67 ± 2.39
Alanine (Ala)	33.43 ± 3.41
Tyrosine (Tyr)	17.27 ± 1.87
Aspartic acid/asparagine (Asp)	59.41 ± 5.32
Proline (Pro)	21.26 ± 2.04
Total amino acids (TAA)	519.54 ± 51.29

values present the means ± SE

*Animals, diets and experimental procedures*

All surgical and experimental procedures were approved by the Animal Care and Ethics Committee of China Agriculture University. Six lactating Chinese Saanen dairy goats (485 ± 21 g/d of milk, 38 ± 2 kg body weight), were used in a Latin square design. Each experiment lasted for 12 days. All goats were prepared with duodenal fistulas and unilateral skin-covered carotid artery catheters. These procedures were performed under full surgical anaesthesia. Two temporary catheters were inserted into the mammary vein (superficial epigastric vein) 1 day prior to collection of blood samples. One catheter was inserted distally into the mammary vein and used for infusing para-aminohippuric acid (PHA, Sigma Chemical Co., USA). The other was inserted proximally and used for collecting

blood samples (Wang et al., 2007). Catheters were kept unobstructed by flushing once daily with a sterile heparin-saline (200 U/ml) solution.

Goats were placed individually into metabolism crates and allowed 14 days to adapt. Feed was delivered in 12 equal portions at 2 h intervals, and water was available *ad libitum*. The goats were hand-milked twice daily (07.00 and 19.00 h).

The composition of the diet is shown in Table 2. It comprised 60% forage and 40% concentrate feed, including vitamin and mineral mixes. Daily feed refusals were collected and weighed.

Table 2. Ingredients and nutrient levels of the experiment diet in dry matter

Ingredients, %		Nutrient levels	
Lucerne hay	40	DM, %	90.06
Chinese wildrye hay	20	ME, MJ/kg <sup>2</sup>	8.86
Maize	28	MP, g/kg DM <sup>2</sup>	50.12
Wheat bran	9	NDF, %	45.11
Soyabean meal	2	Ca, %	0.75
Dicalcium phosphate	0.2	P, %	0.34
Salt	0.4		
Minerals and vitamins <sup>1</sup>	0.4		

<sup>1</sup> contained, IU/kg: vit. A 15,000,000, vit. D 15,000,000; mg/kg: vit. E 3000, Mn 4000, Zn 6000, Fe 2000, Cu 3000, I 150, Se 100 and Co 40; <sup>2</sup>MP - metabolizable protein; ME and MP were calculated values<sup>3</sup>. Other nutrient levels are measured values

The animals were divided into three experimental groups: 1. control, duodenal infusion of 0.9% sodium chloride solution (SCS) 700 ml/d; 2. SSP treatment, duodenal infusion of 60 g/d SSP and 3. FAA treatment; FAA were the same as the AA composition and content in 60 g of SSP. SSP and FAA were dissolved in 700 ml/d of 0.9% sodium chloride solution, respectively. Infusions were conducted continuously using a peristaltic pump. Each infusion period lasted for 12 days. On the last day, PAH was infused into the mammary vein catheter. After 1 h of infusing PAH, blood samples were collected from the carotid artery and mammary vein at 1 h intervals into centrifuge tubes containing heparin-saline. Mammary plasma flow (MPF) was measured by downstream dilution of PAH in the mammary vein (Katz and Bergman, 1969). All goats were kept standing during the blood sampling period. On the last day of each experimental period, the milk was weighed, and milk samples were taken for protein and fat content analyses.

### *Analytical techniques*

Plasma was immediately obtained by centrifugation at 3000 g for 15 min at 4°C and was stored at -20°C until further analysis. Plasma was thoroughly mixed with 1 M perchloric acid (1:1 vol/vol), centrifuged at 1500 g at 4°C for 15 min. The supernatant was re-centrifuged under the same conditions to remove any

residual protein. The supernatant was then neutralized (pH 7-8) by adding 2 M  $K_2CO_3$ , and allowed to stand for 2 h at 4°C before the precipitated perchlorate salt was removed by centrifugation as described above (Backwell et al., 1997). The samples were analysed for total amino acid (TAA, FAA+ peptide-bound AA) by an amino acid analyzer (SYKAM, S433D; Munich, Germany). Milk AA were analysed by the same equipment after removal of milk fat and subsequent hydrolysis (Backwell et al., 1997).

Total RNA was extracted from the mammary gland tissue using the Trizol kit according to the manufacture's protocol, quantified spectrophotometrically at 260/280 nm, then stored at -80°C (Sambrook, 2000).

The cDNA synthesis reactions were performed in a total volume of 20  $\mu$ l containing 1  $\mu$ g RNA, 5 $\times$ M-MLV buffer 4  $\mu$ l, 10 mmol dNTP, 20 U Rnasein, M-MLV 100U, and 20 pmol Oligo dT. The reaction mixture was incubated at 20°C for 5 min, then at 42°C for 60 min and at 70°C for 5 min. The cDNA samples were stored -20°C after cooling (Sambrook, 2000).

The primers listed in Table 3 were designed according to the gene sequences published in GenBank (Mabjeesh, 2000). The number of molecules of mRNA present for each gene of interest per nanogram of total RNA starting

Table 3. Sequence of oligonucleotide primers used for real-time PCR

Name	Oligo	Primer sequence	Predictd size, bp	GenBank accession
APN	Forward Primer	5'- CTTCTCCAGCAGCAACA-3'	226	AJ304432
	Reverse Primer	5'- GCGTCCACAGCCATTACA -3'		
GAPDH	Forward Primer	5'- GCAAGTTCACGGCACAG-3'	249	AJ431207
	Reverse Primer	5'- GGTTACGCCCCATCACAA -3'		

template was determined by real-time PCR (Prism 7900, ABI, Foster City, USA; Gilbert, 2007). The cDNA was diluted to  $10^4$ -10 molecules before addition to the PCR medium containing primers and the SYBR green master mix (ABI, Cat # 4309155, Foster City, USA). This was followed by product melting to confirm single PCR products. Amplification of cDNA samples was performed under the following conditions: 15 min denaturation at 95°C, 40 cycles of 15 s at 94°C, 30 s at 55°C and 45 s at 72°C, and 1 cycle at 72°C for 10 min. The products of real-time PCR were examined on a 1.5% agarose gel. The relative quantity of targeted mRNA expression was analysed using GAPDH as the house-keeping gene. The cDNA fragments of the APN gene were obtained from the mammary gland. Their nucleotide sequences were compared with the published sequence.

*Calculations and statistical analyses*

Mammary plasma flow (MPF) was determined by calculating the arteriovenous difference between PAH infusion rates as follows (Wang, 2007):

$$\text{MPF} = 2 \times I / (\text{PAH}_{\text{mv}} - \text{PAH}_{\text{a}})$$

where:  $I$  - the infusion rate of PAH into the mammary vein (g/h),  $\text{PAH}_{\text{mv}}$  and  $\text{PAH}_{\text{a}}$  - PAH concentration in the plasma of the mammary vein and carotid artery (g/l).

Mammary uptake (MU) of AA was calculated as:

$$\text{MU} = \text{MPF} \times \text{AA}_{(\text{M-A})}$$

where: MU - the net mammary uptake of AA (mg/h), MPF - the mammary plasma flow, A and M - concentration of AA in the artery and mammary vein (mg/l).

The mammary uptake to milk output ratio of AA ( $U/O$ ) was calculated by dividing the amount supplied into the mammary gland by the amount of individual AA secreted into milk per day:

$$U/O = \text{mammary AA uptake per day (g)} / \text{amount of AA secreted into milk per day (g)}$$

The data were tested for treatment effects using the GLM procedure of SAS (1998). The multiple comparisons among treatment means were performed by the Duncan method.

**RESULTS**

Milk yield, milk fat yield, and milk fat content were not significantly changed by FAA infusion (Table 4). Compared with the control treatment, significant increases in milk protein yield and milk protein content after FAA infusion were observed ( $P < 0.05$ ). Milk fat yield and milk fat content were significantly decreased, however, by SSP infusion ( $P < 0.05$ ). Milk lactose content and yield were not affected by treatment ( $P > 0.05$ ).

The concentration of most AA in arterial plasma was increased in goats infused with SSP and FAA compared with the controls (Table 5). Total essential AA (EAA) concentrations in goats infused with FAA were numerically greater than those infused with SSP and these exceeded that of controls. Differences in the concentration of other EAA between SSP and FAA infusion treatments were significant ( $P < 0.05$ ) except for Val, Ile and Met. A similar pattern of differences was observed in the concentration of non-essential AA (NEAA). The concentrations

Table 4. Effect of SSP and FAA infusion on lactation performance of goats

Item	SCS <sup>3</sup>	SSP <sup>4</sup>	FAA <sup>5</sup>	SEM
DMI, kg/d	1.17	1.01	1.08	
MP, g/d <sup>1</sup>	58.67	79.99	80.93	
from diet	58.67	51.91	52.85	
from AA infusion <sup>2</sup>	0	28.08	28.08	
Milk yied, kg/d	0.48	0.51	0.50	0.01
Milk protein yield, g/d	23.92 <sup>a</sup>	29.23 <sup>ab</sup>	35.58 <sup>b</sup>	2.68
Milk protein, %	5.04 <sup>a</sup>	5.76 <sup>a</sup>	7.23 <sup>b</sup>	0.59
Milk fat yield, g/d	39.35 <sup>a</sup>	32.90 <sup>b</sup>	40.09 <sup>a</sup>	1.65
Milk fat, %	8.27 <sup>a</sup>	6.51 <sup>b</sup>	8.12 <sup>a</sup>	0.34
Milk lactose yield, g/d	23.83	23.90	24.25	0.72
Milk lactose, %	5.00	4.75	4.90	0.28

<sup>1</sup> MP - metabolizable protein; <sup>2</sup> assuming the digestibility of the FAA and SSP duodenally infused was 90%; <sup>3</sup> SCS - 0.9% sodium chloride solution; <sup>4</sup> SSP - soyabean small peptides; <sup>5</sup> FAA- free amino acids; letters in the same row differ; <sup>a,b</sup> - P<0.05; <sup>A,B</sup> - P<0.01

Table 5. Arterial concentration of AA in plasma of goats infused SSP and FAA, mg/l

Amino acids	SCS <sup>1</sup>	SSP <sup>2</sup>	FAA <sup>3</sup>	SEM
MPF <sup>4</sup> , l/h	6.99	7.04	7.02	0.02
Thr	39.0 <sup>A</sup>	52.1 <sup>A</sup>	70.2 <sup>B</sup>	3.3
Val	75.3 <sup>a</sup>	101.6 <sup>b</sup>	98.9 <sup>b</sup>	2.1
Phe	44.1 <sup>A</sup>	55.4 <sup>A</sup>	73.7 <sup>B</sup>	3.2
Lys	56.6 <sup>A</sup>	83.6 <sup>B</sup>	110.1 <sup>C</sup>	1.9
Ile	42.9 <sup>a</sup>	65.0 <sup>b</sup>	76.23 <sup>b</sup>	3.6
Leu	83.8 <sup>a</sup>	103.4 <sup>a</sup>	133.5 <sup>b</sup>	6.2
His	56.1 <sup>A</sup>	65.3 <sup>A</sup>	97.9 <sup>B</sup>	2.8
Arg	64.1 <sup>a</sup>	70.8 <sup>a</sup>	92.2 <sup>b</sup>	5.4
Met	12.3 <sup>a</sup>	17.3 <sup>b</sup>	17.5 <sup>b</sup>	1.4
Asp/Asn	74.2 <sup>a</sup>	86.6 <sup>a</sup>	126.8 <sup>b</sup>	5.1
Ser	43.7 <sup>a</sup>	65.7 <sup>b</sup>	70.6 <sup>b</sup>	3.3
Glu/Gln	157.4 <sup>a</sup>	179.1 <sup>a</sup>	220.3 <sup>b</sup>	8.3
Gly	78.6 <sup>a</sup>	99.7 <sup>b</sup>	104.1 <sup>b</sup>	3.1
Ala	47.4 <sup>A</sup>	57.8 <sup>B</sup>	80.9 <sup>C</sup>	1.9
Tyr	29.7 <sup>a</sup>	34.6 <sup>a</sup>	49.0 <sup>b</sup>	2.5
Pro	39.5 <sup>a</sup>	59.3 <sup>b</sup>	60.0 <sup>b</sup>	1.9
TEAA <sup>5</sup>	474.3 <sup>A</sup>	614.5 <sup>B</sup>	770.4 <sup>C</sup>	19.9
TNEAA <sup>6</sup>	470.6 <sup>A</sup>	582.8 <sup>B</sup>	711.8 <sup>C</sup>	17.8

<sup>1</sup> SCS - 0.9% sodium chloride solution; <sup>2</sup> SSP - soyabean small peptides; <sup>3</sup> FAA- free amino acids; <sup>4</sup> MPF - mammary plasma flow; <sup>5</sup> TEAA - total essential AA; <sup>6</sup> TNEAA - total non-essential AA; letters in the same row differ; <sup>a,b</sup> - P<0.05; <sup>A,B</sup> - P<0.01

of total NEAA measured after FAA infusion were the highest among treatments (P<0.05), and the concentration of Asp, Glu, Ala and Tyr were significantly different (P<0.05) between SSP and FAA infusion treatments.

Mammary uptake of EAA was increased by SSP and FAA infusion (Table 6). The mammary uptake of Thr, Val, Phe, Lys, Ile, Leu, Met and His after FAA infusion treatment was higher (P<0.05), and only that of Arg was lower compared with SSP infusion. With the exception of Asp, Glu and Gly, the mammary uptakes

Table 5. Arterial concentration of AA in plasma of goats infused SSP and FAA, mg/l

Amino acids	SCS <sup>1</sup>	SSP <sup>2</sup>	FAA <sup>3</sup>	SEM
MPF <sup>4</sup> , l/h	6.99	7.04	7.02	0.02
Thr	39.0 <sup>A</sup>	52.1 <sup>A</sup>	70.2 <sup>B</sup>	3.3
Val	75.3 <sup>a</sup>	101.6 <sup>b</sup>	98.9 <sup>b</sup>	2.1
Phe	44.1 <sup>A</sup>	55.4 <sup>A</sup>	73.7 <sup>B</sup>	3.2
Lys	56.6 <sup>A</sup>	83.6 <sup>B</sup>	110.1 <sup>C</sup>	1.9
Ile	42.9 <sup>a</sup>	65.0 <sup>b</sup>	76.23 <sup>b</sup>	3.6
Leu	83.8 <sup>a</sup>	103.4 <sup>a</sup>	133.5 <sup>b</sup>	6.2
His	56.1 <sup>A</sup>	65.3 <sup>A</sup>	97.9 <sup>B</sup>	2.8
Arg	64.1 <sup>a</sup>	70.8 <sup>a</sup>	92.2 <sup>b</sup>	5.4
Met	12.3 <sup>a</sup>	17.3 <sup>b</sup>	17.5 <sup>b</sup>	1.4
Asp/Asn	74.2 <sup>a</sup>	86.6 <sup>a</sup>	126.8 <sup>b</sup>	5.1
Ser	43.7 <sup>a</sup>	65.7 <sup>b</sup>	70.6 <sup>b</sup>	3.3
Glu/Gln	157.4 <sup>a</sup>	179.1 <sup>a</sup>	220.3 <sup>b</sup>	8.3
Gly	78.6 <sup>a</sup>	99.7 <sup>b</sup>	104.1 <sup>b</sup>	3.1
Ala	47.4 <sup>A</sup>	57.8 <sup>B</sup>	80.9 <sup>C</sup>	1.9
Tyr	29.7 <sup>a</sup>	34.6 <sup>a</sup>	49.0 <sup>b</sup>	2.5
Pro	39.5 <sup>a</sup>	59.3 <sup>b</sup>	60.0 <sup>b</sup>	1.9
TEAA <sup>5</sup>	474.3 <sup>A</sup>	614.5 <sup>B</sup>	770.4 <sup>C</sup>	19.9
TNEAA <sup>6</sup>	470.6 <sup>A</sup>	582.8 <sup>B</sup>	711.8 <sup>C</sup>	17.8

<sup>1</sup> SCS - 0.9% sodium chloride solution; <sup>2</sup> SSP - soyabean small peptides; <sup>3</sup> FAA- free amino acids;

<sup>4</sup> MPF - mammary plasma flow; <sup>5</sup> TEAA - total essential AA; <sup>6</sup> TNEAA - total non-essential AA; letters in the same row differ; <sup>a,b</sup> - P<0.05; <sup>A,B</sup> - P<0.01

Table 6. Mammary uptake of AA from plasma of goats infused SSP and FAA, mg/h

Amino acids	SCS <sup>1</sup>	SSP <sup>2</sup>	FAA <sup>3</sup>	SEM
Thr	45.6 <sup>A</sup>	78.6 <sup>B</sup>	162.8 <sup>C</sup>	6.2
Val	67.8 <sup>A</sup>	145.6 <sup>B</sup>	1811 <sup>C</sup>	4.6
Phe	40.7 <sup>A</sup>	66.1 <sup>A</sup>	132.9 <sup>B</sup>	7.0
Lys	25.1 <sup>A</sup>	137.5 <sup>B</sup>	236.6 <sup>C</sup>	5.8
Ile	47.2 <sup>A</sup>	84.5 <sup>A</sup>	171.7 <sup>B</sup>	7.8
Leu	98.5 <sup>a</sup>	158.6 <sup>a</sup>	281.6 <sup>b</sup>	18.5
His	10.5 <sup>A</sup>	25.2 <sup>A</sup>	183.6 <sup>B</sup>	4.2
Arg	8.7 <sup>A</sup>	116.8 <sup>B</sup>	32.3 <sup>C</sup>	4.1
Met	20.6 <sup>a</sup>	32.9 <sup>b</sup>	34.7 <sup>b</sup>	2.7
Asp/Asn	28.3 <sup>a</sup>	22.3 <sup>a</sup>	243.2 <sup>b</sup>	3.6
Ser	35.2 <sup>A</sup>	100.6 <sup>B</sup>	156.8 <sup>C</sup>	10.2
Glu/Gln	174.8 <sup>a</sup>	147.5 <sup>a</sup>	312.0 <sup>b</sup>	13.5
Gly	102.7 <sup>a</sup>	109.2 <sup>a</sup>	58.8 <sup>b</sup>	5.3
Ala	-6.4 <sup>A</sup>	40.7 <sup>B</sup>	129.7 <sup>C</sup>	2.9
Tyr	30.1 <sup>A</sup>	49.5 <sup>A</sup>	89.4 <sup>B</sup>	4.0
Pro	-17.8 <sup>A</sup>	44.3 <sup>B</sup>	6.1 <sup>C</sup>	2.9
TEAA <sup>4</sup>	365.0 <sup>A</sup>	829.78 <sup>B</sup>	1384.2 <sup>C</sup>	62.6
TNEAA <sup>5</sup>	382.8 <sup>A</sup>	518.4 <sup>A</sup>	957.8 <sup>B</sup>	32.9

<sup>1</sup> SCS - 0.9% sodium chloride solution<sup>2,3,4,5</sup> explanation see Table 5; <sup>a,b</sup> - P<0.05; <sup>A,B</sup> - P<0.01

of most NEAA were increased when the goats were infused with SSP and FAA through duodenal fistulas. The uptakes of Asp, Ser, Glu, Ala and Tyr were higher in the FAA treatment (P<0.05), whereas that of Pro was lower than in the SSP



treatment group. The mammary uptake of TEAA and TNEAA was the highest following the FAA treatment. The mammary uptake of total AA (TEAA+TNEAA) in the FAA group was 55.2 g/d, thus being increased by 70.8% compared with that of the SSP group.

The U/O ratio of all EAA was increased by SSP and FAA infusions ( $P < 0.01$ ), except for the U/O ratio of Met ( $P > 0.05$ ) (Table 7). Most EAA were taken up in

Table 7. Effect of SSP and FAA on mammary uptake to milk output ratio of AA

Amino acid	SCS <sup>1</sup>	SSP <sup>2</sup>	FAA <sup>3</sup>	SEM
Thr	0.70 <sup>A</sup>	1.26 <sup>B</sup>	2.01 <sup>C</sup>	0.07
Val	0.65 <sup>a</sup>	1.63 <sup>b</sup>	1.78 <sup>b</sup>	0.06
Phe	0.66 <sup>A</sup>	1.14 <sup>B</sup>	1.85 <sup>C</sup>	0.06
Lys	0.28 <sup>A</sup>	1.31 <sup>B</sup>	1.97 <sup>C</sup>	0.06
Ile	0.59 <sup>A</sup>	1.30 <sup>B</sup>	2.40 <sup>C</sup>	0.11
Leu	0.64 <sup>A</sup>	1.25 <sup>B</sup>	1.95 <sup>C</sup>	0.10
His	0.20 <sup>A</sup>	0.71 <sup>A</sup>	4.01 <sup>B</sup>	0.12
Arg	0.22 <sup>A</sup>	2.91 <sup>B</sup>	0.66 <sup>C</sup>	0.08
Met	0.70 <sup>a</sup>	0.75 <sup>a</sup>	0.25 <sup>b</sup>	0.02
Asp/Asn	0.27 <sup>a</sup>	0.22 <sup>a</sup>	2.22 <sup>b</sup>	0.03
Ser	0.50 <sup>A</sup>	1.52 <sup>B</sup>	2.06 <sup>C</sup>	0.11
Glu/Gln	0.44 <sup>a</sup>	0.54 <sup>a</sup>	1.04 <sup>b</sup>	0.04
Gly	4.28 <sup>A</sup>	4.89 <sup>A</sup>	1.87 <sup>B</sup>	0.16
Ala	-0.09 <sup>A</sup>	0.87 <sup>B</sup>	2.57 <sup>C</sup>	0.06
Tyr	0.43 <sup>A</sup>	0.85 <sup>B</sup>	1.42 <sup>C</sup>	0.05
Pro	-0.13 <sup>A</sup>	0.30 <sup>B</sup>	0.05 <sup>C</sup>	0.03
TEAA <sup>4</sup>	0.50 <sup>A</sup>	1.33 <sup>B</sup>	1.89 <sup>C</sup>	0.10
TNEAA <sup>5</sup>	0.55 <sup>A</sup>	0.91 <sup>B</sup>	1.58 <sup>C</sup>	0.06

<sup>1</sup> SCS - 0.9% sodium chloride solution; <sup>2,3,4,5</sup> explanation see Table 5; <sup>a,b</sup> -  $P < 0.05$ ; <sup>A,B</sup> -  $P < 0.01$

excess when infused in SSP and FAA, but their ratio in the SSP treatment were lower than those in the FAA treatment, apart from Met. The ratios of all NEAA varied among the treatments, especially that for Gly. Its U/O ratio in the control treatment was 4.28 for milk output, and 1.87 for the FAA treatment. In the SSP and FAA groups, the uptake of AA (32.4 vs 55.2 g/d) was higher than the milk protein output (29.3 vs 35.6 g/d). The U/O of TEAA and TNEAA were significantly different ( $P < 0.01$ ), and the uptake of TEAA was excessive compared with secretion in milk in the SSP and FAA infusion groups.

The abundance of APN mRNA was 38.28 in the SSP infusion group, and significantly lower at 2.83, 5.28 in the control and FAA infusion groups, respectively (SEM: 1.13).

## DISCUSSION

The infusion of SSP and FAA increased the milk protein content by 5.8 and 7.2%, respectively, and yield by 29 and 36 g/d, respectively (both not significant for SSP), suggesting an increase in the substrate for milk protein synthesis. These results differ, however, from those of previous studies in which there was no significant increase in milk protein in response to AA and dietary protein supplementation (Choung and Chamberlain, 1993; Bequette et al., 1996b). Infusing goats in the early stages of lactation with an AA mixture that did not include leucine did not cause an increase in milk protein output (Bequette et al., 1996a). The milk protein percentage and yield in the FAA group was higher, however, than those in the SSP group, which suggests the FAA was more effective for milk protein synthesis.

In the present study, the arterial plasma concentrations of most EAA were increased and showed significant differences from values in the control group, depending on whether SSP or FAA were infused ( $P < 0.05$ ). Significant differences were observed especially for arterial concentrations of Thr, Phe, Lys and His ( $P < 0.01$ ). MPF was not affected by SSP and FAA infusion ( $P > 0.05$ ). AA uptake by the mammary gland is a multifaceted process involving changes in MPF and plasma AA concentrations. Since MPF was not significantly altered in order to meet the AA requirement for milk protein synthesis, a change in plasma AA might have been the driving variable. The changes in EAA arterial concentrations varied in SSP and FAA infusions, suggesting that the availability of individual AA that could be absorbed from the gastrointestinal tract by the mammary gland was variable, and might be related to the need for milk protein synthesis and degradation in the liver (Derrig et al., 1974; Guinard et al., 1994). In the SSP and FAA treatment, the arterial plasma concentration of AA (TEAA+TNEAA) was similar, which suggested that the absorption of FAA and small peptides in the small intestine was not significantly different.

Mammary uptake of most EAA generally exceeds the quantities excreted in the milk. Some of those AA are used as milk protein precursors, some are transformed into other AA, e.g., Pro from Arg (Bruckental et al., 1991), or are used for other metabolic functions (Tagari et al., 2004). In addition, some AA, such as branched-chain AA (Val, Leu and Ile), are taken up in excessive amounts by mammary tissue and the excesses are oxidized (Roets et al., 1979; Bequette et al., 1996a,b, 1998, 1999; Mabweesh et al., 2000a). In the control group of the present study, the mammary gland uptake of AA (TEAA+TNEAA) was 18 g/d ( $0.75 \times 24$ ), and was lower than the 24 g/d AA in milk protein output. The supply of AA for milk protein synthesis was therefore deficient by 24% in the control treatment, and lack of AA might be compensated for by uptake of circulating peptide-bound AA (Tagari et al., 2004, 2008), suggesting that peptide-bound AA could be used by

the mammary gland. In the SSP and FAA groups, the uptake of AA (32 vs 55 g/d) was higher than the milk protein output (29 vs 36 g/d). These results suggest that in order to balance the net requirements, the mammary gland is able to change the proportion of AA derived from the free AA or peptide pools in the blood, depending on supply. Mammary uptake of TEAA in FAA treatment was higher, however, than in the SSP treatment, which suggests that FAA was more effective for milk protein synthesis.

APN could be one of the mechanisms involved in acquiring substrates to be used for milk protein synthesis and secretion. The abundance of its mRNA should be sensitive to any metabolic challenge at the substrate level, and it might be one of the peptidases utilizing small peptides in the mammary gland (Shennan et al., 1996, 1998). It is imbedded on the basolateral side of parenchymal cells, and is an exopeptidase that cleaves N-terminal AA from peptides (Mabjeesh et al., 2005). In the current study, we infused SCS, SSP and FAA, respectively. APN mRNA expression in the mammary gland was significantly increased by SSP infusion, which may cause an increase in the concentration of small peptides in the mammary gland, suggesting that the activity of APN was related to utilization of small peptides by this gland.

## CONCLUSIONS

Free amino acids (FAA) infused into the duodenum improved milk protein synthesis, while this effect was smaller and not significant for soyabean small peptides (SSP). FAA supplied at the same amount of individual AA as SSP was, therefore, more effective. We assume that absorption of small peptides in the mammary gland was only a complementary mechanism for milk protein synthesis. The activity of aminopeptidase N in mammary gland seems to be related to utilization of small peptides.

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