Morphology, proliferation, and ribonucleic acid and fractional protein syntheses in the small intestinal mucosa of young goats fed soy protein-based diets with or without amino acid supplementation

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ABSTRACT

The study was designed to examine whether feeding soy protein isolate as partial replacement of casein (CN) affects jejunal protein synthesis and whether effects may be ameliorated by supplementation of those AA known to be at lower concentrations in soy protein isolate than in CN. Goat kids (14 d) were fed comparable milk protein diets, in which 50% of the crude protein was CN (CAS), soy protein isolate (SPI), or soy protein isolate supplemented with AA (SPIA) for 43 d ($n = 8$ per group). On d 42, plasma concentrations of protein, urea, and AA were measured before and after morning feeding. In the morning of d 43, [¹⁵N]RNA from yeast [13 mg/kg of body weight (BW)] was given with the diet to measure the reutilization of dietary RNA precursors for mucosal RNA biosynthesis. Four hours later, an oral dose of L-[1-¹³C]leucine (180 mg/kg of BW) was administered and blood samples were collected between -15 and +45 min relative to tracer administration for analysis of plasma ¹³C α -ketoisocaproic acid and ¹³C recovery in blood CO₂. Kids were killed 60 min after the tracer application, and jejunal tissue was collected to determine mucosal morphology, cell proliferation, enzyme activities, RNA synthesis, and fractional protein synthesis rate. Plasma protein concentrations were higher in CAS than in SPI and SPIA. Plasma concentrations of Thr were higher in CAS than in SPI and SPIA, and those of Met were lower in SPI than in CAS and SPIA. In mid-jejunum, villus circumferences were higher in CAS than in SPI and SPIA, and villus height and villus height:crypt depth ratio were higher in CAS than in SPI. In mid-jejunum, mucosal protein concentrations were higher in CAS than in SPI and SPIA and mucosal activities of aminopeptidase

N tended to be higher in CAS than in SPI, whereas activities of dipeptidyl peptidase IV tended to be lower in SPI than in SPIA. Activities of 5' nucleotidase and xanthine oxidase were lower in CAS than in SPI. The ¹³C recovery in blood CO₂ tended to be higher in SPI than in CAS. In mid-jejunum, ¹⁵N enrichment of RNA tended to be higher in CAS than in SPI, and ¹³C enrichment of protein-bound Leu was higher in SPI than in CAS. In mid-jejunum, the fractional protein synthesis rate tended to be higher in SPI than in CAS. Our results revealed changes in intestinal growth after soy protein feeding that were associated with effects on intestinal RNA and protein synthesis but that were not ameliorated by AA supplementation.

Key words: goat kid, soy protein, jejunum, protein synthesis

INTRODUCTION

In preruminants, feeding soy protein instead of milk protein leads to an alteration in the intestinal mucosal structure (Montagne et al., 1999) and impairs intestinal absorptive ability, shown by xylose absorption tests (Seegraber and Morill, 1982), which is partially caused by an overall deficiency of indispensable AA (IAA; Kanjanaputhipong, 1998). Supplementation of IAA to soy protein seems to ameliorate mucosal growth retardation (Schönhusen et al., 2010) and probably stimulates mucosal protein synthesis in the gut (Deutz et al., 1998; Stoll et al., 1998). Studies by Nieto et al. (1994) in growing chicken indicated that supplementation of Leu to soy protein stimulates protein synthesis in the jejunum. On the other hand, dietary deficiency of Thr reduces the synthesis of mucosal protein and mucins in the jejunum of young pigs (Faure et al., 2005; Wang et al., 2007). We have demonstrated that soy protein with Thr, Val, Ile, Leu, His, Lys, and Met changes jejunal proteins involved in processes related to cytoskeleton generation and energy metabolism in young goats (Schönhusen et al., 2010).

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In the jejunum of young calves, the rate of protein synthesis was negatively correlated with villus height:crypt depth ratio and positively with cell proliferation in crypts (Rufibach et al., 2006). Grant et al. (1989) showed an association between reduced mucosal protein:RNA ratio and decreased efficiency of translational processes in the small intestine of calves fed soy protein diets. We recently found a reduced protein:RNA ratio and altered RNA metabolism in the small intestine of goat kids that were fed soy protein instead of milk protein; however, AA supplementation did not counteract a reduced translational activity due to soy feeding (Schönhusen et al., 2010). Burrin et al. (1991) stated that alterations in the rate of protein synthesis are a result of changes in cellular mechanisms involving the numbers of ribosomes per cell, the activity of these ribosomes, and translational capacity and efficiency. Besides, it seems likely that the molecular form in which dietary AA are ingested, either as free or protein-derived AA, affects their absorption kinetics and oxidation as well their peripheral and splanchnic availability for protein synthesis (Metges et al., 2000; Daenzer et al., 2001).

We hypothesized that soy protein isolate as partial replacement of CN in milk diets impairs the mucosal protein synthesis in the jejunum of young goats and that a supplementation of IAA to soy protein isolate would ameliorate this soy effect. Because changes in protein metabolism are closely associated with changes in RNA metabolism in mucosal cells, we examined treatment effects on protein and RNA synthesis by using a dual label technique. In contrast to our earlier studies, we fed soy protein isolate instead of a commercial soy protein product to investigate effects caused by AA limitation in more detail, because soy protein isolate is almost free of antigenic and immunoreactive substances that interfere with its digestion and absorption (Lallès et al., 1995; Montagne et al., 2001).

MATERIALS AND METHODS

Animals, Diets, and Experimental Procedures

Animals were treated in accordance with the guidelines for the use of animals as experimental subjects of the State Government of Mecklenburg-West Pomerania, Germany (LALL M-V/TSD/7221.3-2.1-017/05).

Twenty-four male kids (German White dairy goat) were purchased from a goat farm at the age of 12 d. All kids were delivered naturally and suckled colostrum and milk before they were removed from their dams. Kids were weighed upon receipt at the Research Institute (FBN, Dummerstorf, Germany) ($BW\ 6.1 \pm 0.2$ kg) and assigned randomly by age and BW to the

3 dietary treatments ($n = 8$ per group). Groups were housed in individual boxes (3.5×4 m) at an ambient temperature of about 15°C with free access to fresh water and straw as bedding material. Before starting the study, kids were fed progressively increasing amounts of cow's milk by bottle thrice daily (0700, 1200, and 1600 h). Dietary treatments were started on d 20 of life for a period of 43 d. All 3 experimental diets (Table 1) were based on skim milk powder [26% Regular, Nordmilch eG, Bremen, Germany, 28% CP, 27% crude fat (CF) in DM]. In the control group (CAS), 50% of the milk protein was replaced by CN [acid-precipitated CN, Molkereigenossenschaft Lauingen mbH, Lauingen, Germany; 1.3% crude ash (CA), 97.6% CP, 1.1% CF in DM]. In the second group (SPI), 50% of the milk protein was replaced by soy protein isolate (Supro-901, Interfood Deutschland GmbH, Germany; 4.7% CA, 90.0% CP, 2.5% CF in DM), and whereas in the third group, SPI with AA supplementation (SPIA), 50% of the milk protein was replaced by soy protein isolate (Supro 901) supplemented with AA (L-Thr 2, L-Ser 4, L-Glu 26, L-Val 9, L-Ile 4, L-Leu 9, L-Tyr 8, L-Phe 1, L-His 1, L-Lys 9, L-Pro 21, L-Met 7, L-Trp 0.1; g/100 g of AA mixture; Merck, Darmstadt, Germany) known to be at lower concentrations in soy protein isolate than in CN (Table 2). Diets were formulated to be isonitrogenous and isoenergetic. Lactose was added to provide a constant proportion of protein to N-free extracts in all 3 diets. Experimental diets were prepared daily by mixing the dry ingredients with water (1:6 wt/wt). A DMI of $33\text{ g}/(\text{kg of } BW^{0.75} \times \text{d})$ was provided and energy and protein were supplied at $0.6\text{ MJ}/(\text{kg of } BW^{0.75} \times \text{d})$ and $11\text{ g}/(\text{kg of } BW^{0.75} \times \text{d})$, respectively (Bezabih and Pfeffer, 2003). Diets were warmed before feeding and were fed twice daily in equal parts at 0700 and 1650 h by bottle. Kids were weighed weekly before the morning feeding, and DMI was adjusted for BW. Diet consumption was recorded daily, and a 50-g aliquot of each experimental diet was collected daily and stored at -20°C until analysis.

On d 42, blood samples for measurement of plasma total protein and urea concentrations were taken via a jugular vein catheter at 30 and 5 min before and at 5, 10, 15, 20, 30, 40, 60, 90, 120, 180, 240, 300, 360, and 420 min after morning feeding. Blood samples were transferred to Li-heparinate monovettes (Sarstedt, Nürnberg, Germany) and centrifuged ($1,500 \times g$, 15 min at 4°C). Blood plasma was aliquoted and stored at -20°C .

In the morning (0700 h) of d 43, ^{15}N -labeled yeast RNA [$13\text{ mg}/\text{kg BW}$, 90 atom percent (AP) ^{15}N , $n = 6$ per group] was given with half of the daily diet to measure the reutilization of dietary RNA precursors for mucosal RNA biosynthesis as described previously (Schönhusen

Table 1. Ingredients and chemical composition of milk based diets containing CN or soy protein isolate without or with AA supplementation fed to goat kids

Item	Diet ¹		
	CAS	SPI	SPIA
Ingredient, g/kg of DM			
Skim milk powder ²	588	587	585
CN ³	170	—	—
Soy protein isolate ⁴	—	172	139
Lactose monohydrate	242	241	241
AA mixture ⁵	—	—	35
Chemical composition, g/kg of DM			
CP	332	329	329
Crude ash	38	44	42
Crude fat	161	163	166
Nitrogen-free extracts	469	464	463
ME, MJ/kg of DM	16.9	16.9	16.9

¹CAS = milk diet containing acid-precipitated CN (50% of total CP in the diet); SPI = milk diet containing soy protein isolate (50% of total CP in the diet); SPIA = milk diet containing soy protein isolate supplemented with those AA known to be at lower concentrations in soy protein isolate than in CN (50% of total CP in the diet).

²Skim milk powder, 26% Regular, Nordmilch eG, Bremen, Germany.

³Acid-precipitated CN, Molkereigesellschaft Lauingen mbH, Lauingen, Germany.

⁴Soy protein isolate Supro-901, Interfood Deutschland GmbH, Bad Nauheim, Germany; Isoflavone contents: daidzein 516; genistein 1,274 (µg/g of DM).

⁵Composition: L-Thr 2; L-Ser 4; L-Glu 26; L-Val 9; L-Ile 4; L-Leu 9 g; L-Tyr 8; L-Phe 1, L-His 1; L-Lys 9; L-Pro 21; L-Met 7; Trp 0.1 (g/100 g of AA mixture).

et al., 2007). To obtain baseline data, the same amount of unlabeled RNA (from yeast; Serva, Heidelberg, Germany) was administered to 2 kids of each group. Four hours later, a single bolus dose of L-[1-¹³C]leucine (180 mg/kg of BW, 99 AP ¹³C, Chemotrade, Leipzig, Ger-

many, n = 6 per group) was fed together with 100 mL of the diet to measure fractional protein synthesis rate (**FSR**) in jejunum. For baseline data, the same amount of unlabeled Leu was administered to 2 kids of each group. Blood samples were taken via jugular vein cath-

Table 2. Contents of total N and AA of proteins in the experimental diets and quantity of free AA supplemented to soy protein isolate (SPI)

Item	Protein		
	CN ¹	SPI ²	AA supplemented to SPI
Total N, g/100 of DM	15.62	15.37	
AA, g/16 g of N			
Asp	6.57	9.96	
Thr	4.17	3.84	0.4
Ser	5.44	4.56	0.9
Gln + Glu	22.29	15.76	6.6
Gly	1.77	3.85	
Ala	2.95	4.63	
Val	6.29	4.05	2.3
Ile	4.92	3.96	1.0
Leu	9.24	6.90	2.4
Tyr	5.39	3.41	2.0
Phe	5.02	4.92	0.1
His	2.84	2.63	0.2
Lys	8.06	5.81	2.3
Arg	3.67	6.74	
Pro	10.68	5.38	5.3
Cys	0.39	1.30	
Met	2.76	1.04	1.7
Trp	1.18	1.17	0.01

¹Acid-precipitated CN, Molkereigesellschaft Lauingen mbH, Lauingen, Germany.

²Supro-901, Interfood Deutschland GmbH, Bad Nauheim, Germany.

eter at -15, -5, 5, 15, 30, and 45 min relative to the L-[1-¹³C]leucine administration. All blood samples were transferred to Li-heparinate monovettes (Sarstedt) held on ice. Whole blood was used immediately for the determination of ¹³C enrichments in blood CO₂. After centrifugation plasma was aliquoted and stored at -20°C for analysis of ¹³C enrichments of α-ketoisocaproic acid (α-KIC). Plasma free AA concentrations were measured at 45 min after L-[1-¹³C]leucine administration. One hour before slaughtering, kids were intravenously injected with 5-bromo-2'-deoxyuridine (BrdU, 100 mg dissolved in 5 mL of PBS; Roche Diagnostics GmbH, Mannheim, Germany) to measure cell proliferation in intestinal mucosa cells.

Kids were killed by stunning using a captive bolt pistol and bled from the carotid arteries. The abdominal cavity was immediately opened and the jejunum was removed and divided into 3 equal segments (proximal, medial, and distal). These segments were rinsed free with ice-cold saline (0.9% NaCl, wt/vol). Tissue sections (1 cm²) from each jejunal segment were cut and transferred into formaldehyde solution (4%, wt/vol) for evaluation of intestinal morphology. For the analyses of intestinal cell proliferation, a tissue sample (1 cm²) of each jejunal segment was stuck onto a piece of cork, frozen in liquid nitrogen, and stored at -80°C until analysis. Mucosal tissue was harvested from the remaining jejunal segments (proximal, medial, distal) by scraping with a glass slide, frozen in liquid N, ground, and stored at -80°C.

Analytical Procedures

Feed. Dry matter, CA, ether extract, and CF of the dried dietary components and experimental diets were determined following the Weender standard procedure (Naumann and Bassler, 1993). A combustion analyzer (CNS-2000, Leco, St. Joseph, MI) was used to determine total N content, which was multiplied by 6.25 to obtain CP. Lactose concentration was measured by the β-galactosidase method using a commercial kit (no. 10 176 303 035, R-Biopharm AG, Darmstadt, Germany). Dietary AA concentrations were measured by liquid ion-exchange chromatography as described previously (Hennig et al. 2004). Total RNA in diets was determined after salt extraction (Schönhusen et al., 2007). Soy protein isolate was analyzed for contents of daidzein and genistein by HPLC after acid hydrolysis and extraction (Degen et al., 2002).

Plasma Protein, Urea, and Free AA Concentration. Total protein concentration in plasma was determined by the Biuret test (Kit LT-GL 0103; LT-

SYS, Labor und Technik Lehmann, Berlin, Germany). For determination of plasma urea concentration, a UV method was used (kit no. LT-UR 0010, LT-SYS, Labor und Technik Lehmann).

Concentrations of free AA in plasma were measured after deproteinization with sulfosalicylic acid (35%, wt/vol) by ion-exchange chromatography using a 0.3 M lithium buffer and a physiological AA standard solution (A9906, Sigma-Aldrich, St. Louis, MO) as internal standard on a Biochrom 20 Plus Amino Acid Analyzer (Biochrom Ltd., Cambridge, UK) using a 50 × 4.6 mm column.

Histomorphometry and Cell Proliferation in Jejunal Mucosa. Morphometric indices (circumference and height of villi, depth of crypts) of jejunal tissue sections were determined as described (Kuhla et al., 2007). Cell proliferation was measured by BrdU labeling (Roche Diagnostics GmbH; Matsuura and Suzuki, 1997). Jejunal tissue was frozen in liquid N and cryosectioned (6 μm, Leica CM3050 Cryostat Microtome; Leica, Nussloch, Germany); 6 slices per jejunal segment (proximal, medial, distal) were obtained. Sections were fixed in paraformaldehyde solution (4%, wt/vol, Roti-Histofix, Roth, Karlsruhe, Germany) for 20 min, washed 2 times in PBS, and permeabilized with PBS containing Triton X-100 (0.1%, wt/vol, Sigma-Aldrich Co.). DNA was denatured with 2 M HCl (60 min at 37°C). After that, nonspecific binding of the secondary antibody was blocked by exposing sections to rabbit serum (10%, wt/vol, no. 16120-099, Invitrogen GmbH, Karlsruhe, Germany) for 15 min. Slides were incubated overnight at 4°C in a humidity chamber with primary antibody (mouse monoclonal anti-BrdU, no. 1170376, Roche Diagnostics AG, Rotkreuz, Switzerland) diluted 1:100 in PBS-Triton X-100 containing rabbit serum (1%, wt/vol). After washing, sections were incubated for 45 min at room temperature in the dark with an Alexa Fluor 488-labeled rabbit anti-mouse IgG secondary antibody (no. A11059, Invitrogen GmbH) diluted 1:500 in PBS-Triton X-100. To visualize unlabeled nuclei, sections were counterstained briefly with propidium iodide (5 μg/mL, no. P4170, Sigma-Aldrich Co.). Slides were rinsed again with PBS and with distilled water. Sections were covered with MobiGlow mounting medium (MobiTec, Göttingen, Germany) and coverslips. Using a computerized image analysis system (ImageC, Imtronic GmbH, Berlin, Germany), the total area of BrdU-labeled crypt cell nuclei (labeled green) as well as the total area of all nuclei (labeled red) were determined for 4 randomly chosen fields per slice and 24 fields per intestinal segment and 72 fields per animal. The area of all detected objects was measured in red

and green channels, respectively. As a measure of cell proliferation, the ratio between BrdU-labeled area and total nuclei area was determined.

Mucosal Protein, RNA, and DNA in Jejunum.

Protein concentration in mucosal tissue (200 mg) was determined using the Coomassie dye binding protein assay (Sigma-Aldrich Co.) with BSA (Fraction V, Sigma-Aldrich) as standard. The RNA was quantified after salt extraction from a 100-mg sample of freeze-dried mucosal tissue (Schönhusen et al., 2007). The DNA was analyzed in freeze-dried mucosal tissue (10 mg) using the GOLDTriFast reagent (peqLab Biotechnologie GmbH, Erlangen, Germany). The total RNA:protein ratio was calculated and used as a measure of translation capacity (Burrin et al., 1997).

Mucosal Enzymes in Jejunum. For analyses of peptidase activity, frozen mucosa tissue was homogenized in ice-cold water (200 mg/mL) and centrifuged ($1,000 \times g$, 5 min at 4°C). Activity of aminopeptidase N (APN, EC 3.4.11.2) was assayed with L-leucyl-p-nitroanilid as substrate (Maroux et al., 1973), and dipeptidyl-peptidase IV (DPP-IV, EC 3.4.14.5) was assayed with glycyl-L-prolyl-p-nitroanilid (Nagatsu et al., 1976). The resulting units (IU) were expressed as nanomoles of p-nitroanilid released per minute at 37°C. Activity of 5'-nucleotidase (5'NT, EC 3.1.3.5) was measured in enterocytes, separated from the jejunal wall by vibration (IKA-Vibrax-VXR, IKA-Werke GmbH Ko.KG, Staufen, Germany). The activity was assayed from the amount of inorganic phosphate from AMP (Ipata, 1967). Phosphate release was determined by using the Malachite Green Phosphate Assay kit (BioAssay Systems, Hayward, CA). One 5'NT IU corresponded to the release of 1 nanomole of phosphate per minute at 37°C. For analysis of xanthine oxidase (XO) activity, frozen intestinal mucosa was homogenized in 0.05 M HEPES buffer (200 mg/mL) and centrifuged ($30,000 \times g$, 20 min at 4°C). Activity of XO was measured as the rate of uric acid production using xanthine as substrate (Furth-Walker and Amy, 1987). The rate of uric acid production was defined as 1 IU of XO producing 1 nanomole of uric acid per minute at 37°C. For all these enzymes, the results were expressed as international units per milligram of proteins. Protein concentration was determined by the method of Bradford (Bradford, 1976).

Plasma α -[1- ^{13}C]Ketoisocaproic Acid and Mucosal [1- ^{13}C]Leucine Enrichments. To determine the in vivo FSR in jejunum, measurements of ^{13}C enrichments of free (intracellular) and protein-bound Leu were performed (Garlick and McNurlan, 1998). Plasma enrichments of the transamination product α -[1- ^{13}C]KIC were used as an indicator of intracellular ^{13}C enrichment. The α -KIC enrichments were measured in a

quinoxalinol tert-butyldimethylsilyl derivative, except that acetonitrile was used instead of pyridine (Metges and Daenzer, 2000). The enrichments of plasma α -KIC were measured by electron-impact GC-MS (70 eV) using a gas chromatograph coupled to a quadrupole mass spectrometer Shimadzu GCMS-QP2010 (Shimadzu Corp., Kyoto, Japan). One microliter was injected in split mode. The analysis of α -KIC was carried out on a temperature-programmed DB 1301 column (50 m $\times$ 0.32 mm ID; 0.52 μm ; J&W Scientific, Folsom, CA; 100°C held for 0.5 min and increased from 100 to 280°C at a rate of 30°C/min); α -KIC eluted at 7 min and was monitored at its base peak m/z 259[M-57] $^{+}$.

To determine the enrichments of [1- ^{13}C]leucine bound in mucosal protein, frozen mucosal samples (~16 mg) were homogenized in TCA (Vortex Genie 2, Scientific Industries Inc., Bohemia, NY). Proteins were separated from acid-soluble AA as described previously by Faure et al. (2002). The measurement of ^{13}C Leu enrichment was performed after derivatization to its *N*-pivaloyl-iso-propyl ester by gas chromatography-combustion-isotope ratio mass spectrometry as described previously (Metges and Daenzer, 2000).

Blood $^{13}\text{CO}_2$ Enrichments. To determine the ^{13}C enrichments in blood CO_2 (representing all acid-volatile CO_2), reflecting the oxidation of ^{13}C Leu, 500 μL of heparinized whole blood was treated with 1 mL of lactic acid (10% wt/wt) and sealed airtight (Junghans et al., 2007). Thereafter, CO_2 released from blood was measured in the headspace by means of gas isotope ratio mass spectrometry (IRMS, DELTA Plus XL, Thermo Quest, Bremen, Germany) coupled with Gas Bench II (Finnigan MAT, Bremen, Germany). Enrichments were calculated as the difference between the enrichments of each blood sample and the baseline abundance (preadministration value).

Mucosal ^{15}N Enrichments. The ^{15}N enrichments in mucosal RNA were determined after separation of RNA from DNA and protein contamination using an elemental analyzer (EA 1108, Fisons Instr. Rodano, Milan, Italy) coupled on-line to a continuous flow interface (CONFlo II, Finnigan MAT), connected to an isotope ratio mass spectrometer (delta S, Finnigan MAT) as described recently (Schönhusen et al., 2007). Enrichments were expressed relative to mucosal baseline abundance. The ^{15}N enrichments of total RNA ingested were 54, 55, and 56 AP in treatments CAS, SPI, and SPIA, respectively. This allowed comparison of the $^{15}\text{N}/^{14}\text{N}$ ratios without correction for the isotopic dilution effect on an equivalent basis relative to intake.

Calculations and Statistical Procedures

The ME content of feed was calculated using digestible nutrients. Digestible nutrients were calculated

from the measured nutrient contents multiplied by the digestibility of nutrients using feed tables (Jentsch et al., 2003).

The FSR (percentage of protein renewed per d, %/d) in mucosa was determined based on the rate of incorporation of L-[1-¹³C]leucine. The FSR was calculated based on the product/precursor relationship mucosa protein-bound, where L-[1-¹³C]leucine (*Sb*) to plasma α-[1-¹³C]KIC considered as a proxy for the intracellular free ¹³C Leu (*Sa*) (Garlick and McNurlan, 1998):

$$\text{FSR} = Sb/Sa \times 100/t,$$

where *t* (min) is the time of incorporation.

Translational efficiency (*k_{RNA}*, g of protein synthesized/d per g of RNA) in mucosa was calculated from the FSR as follows (Lobley et al., 1994):

$$k_{RNA} = \text{FSR}/100/C_s,$$

where *C_s* is total RNA:protein ratio in mucosa.

The ¹³C recovery (¹³C REC) in blood CO₂ (% of ¹³C dose), after an oral bolus dose of [1-¹³C]leucine, is based on a CO₂ production of 1 mmol/min per kg^{-0.75} and can be calculated as follows (Kien, 1989):

$$^{13}\text{C REC} = V(\text{CO}_2)/R_a(\text{CO}_2) \text{ and } R_a(\text{CO}_2) = D/A(^{13}\text{C}),$$

where *V*(CO₂) (mol/min) is the rate of CO₂ produced, *R_a*(CO₂) (mol/min) is the rate of CO₂ appearance, *D* (mol) is the ¹³C dose administered, and *A*(¹³C) (ppm 10⁻⁶) is the area under the ¹³C enrichment (blood CO₂)-time curve (Junghans et al., 2007).

All statistical calculations were performed with procedures of the SAS system, version 9.1.3 (SAS Institute, 2004). Data were expressed as means ± SEM. For the detection of differences among the jejunal segments and the diets, a 2-factorial variance analysis model was not suitable, because estimations of variance for one factor (jejunal segment) showed great differences for nearly all parameters. Therefore, differences between the diets shown for parameters of histomorphometry and cell proliferation, mucosal protein, RNA and DNA concentration and enzyme activity, ¹³C enrichment of protein-bound Leu and ¹⁵N enrichment of RNA, and FSR in jejunum were based on one factor (diet) model by the GLM procedure and were identified by Tukey *t*-test. Differences between feeding groups with 0.05 > *P* < 0.1 were defined as trends and with *P* < 0.05 as significant. Differences among jejunal segments without consideration of feeding groups were analyzed by paired *t*-test of the T TEST procedure. The PROC CORR

procedure of SAS was used to calculate correlations between BrdU-labeling, histomorphometric parameters, tissue ingredient, and FSR.

Treatment and time effects on total protein and urea concentration and ¹³C KIC enrichment in blood plasma were assessed by using the random and repeated methods of PROC MIXED procedure of SAS. The effects of diet, time and their interaction were considered fixed and goat was considered random. Differences were identified by Tukey *t*-test as described above.

RESULTS

Feed Intake, BW, and Health Status

Daily intake of DM [189 ± 6 g/(animal × d)], CP [62.3 ± 2.1 g/(animal × d)], and ME [62.3 ± 2.1 g/(animal × d)] were not different among groups. No difference existed in initial BW (6.1 ± 0.3 kg) and final BW (12.5 kg ± 0.6 kg) among groups. Growth performance and feed efficiency did not differ among feeding groups: ADG was 148 ± 8 g/d and feed efficiency was 786 ± 31 g of ADG/kg of DMI. All kids were healthy and showed no obvious differences in behavior.

Plasma Concentrations of Total Protein, Urea, and Free AA

Fasted plasma total protein concentrations tended to be higher (*P* < 0.1) in CAS than in SPI and were higher (*P* < 0.05) in CAS than in SPIA (Figure 1). Plasma protein concentrations decreased (*P* < 0.001) during the 60-min period after feeding and were higher (*P* < 0.05) in CAS than in SPI and SPIA; the levels remained stable from 60 to 420 min. Plasma urea concentrations were not affected by treatment before feed intake, increased (*P* < 0.001) from 0 to 90 min in CAS, and increased (*P* < 0.001) from 90 to 420 min in SPI and SPIA, and showed a treatment × time interaction (*P* < 0.05; Figure 2). At 7 h after feeding, the concentrations of total free AA and Leu in plasma were not different among groups, but plasma Thr concentrations were higher in CAS than in SPI (*P* < 0.01) and SPIA (*P* < 0.05), whereas plasma Met concentrations were lower (*P* < 0.05) in SPI than in CAS and SPIA (Figure 3).

Morphometric Measurements and Cell Proliferation in Jejunum

In the proximal jejunum, villus circumference (1,471 ± 47 μm), villus height (582 ± 20 μm), and villus height:crypt depth ratio (2.5 ± 0.1) did not differ among dietary treatments, but crypt depth was smaller (*P* <

Figure 1. Concentrations of plasma total protein before (–30 min) and after (up to +420 min) morning feeding in goat kids fed milk-based diets containing CN (CAS) or soy protein isolate without (SPI) or with AA supplementation (SPIA). Values are means $\pm$ SEM, $n = 8$ per group. Fixed effects (P -value) for total protein concentration: diet <0.05 , time <0.001 .

0.05) in CAS than in SPI (224 ± 4 , 246 ± 5 , and 237 ± 6 μm for CAS, SPI, and SPIA, respectively). Villus circumferences in medial and distal jejunum were greater ($P < 0.001$ and $P < 0.05$) in CAS than in SPI and SPIA ($1,491 \pm 31$ and $1,268 \pm 34$; $1,303 \pm 31$ and $1,142 \pm 28$; and $1,372 \pm 21$ and $1,167 \pm 27$ μm for medial and distal jejunum in CAS, SPI, and SPIA, respectively). Villus heights in the medial and distal jejunum were greater ($P < 0.05$) in CAS than in SPI (599 ± 11 and 542 ± 12 ; 553 ± 15 and 476 ± 16 ; and 571 ± 12 and 487 ± 7 μm for CAS, SPI, and SPIA, respectively). There were no group differences in crypt depth in medial (225 ± 5 μm) and distal (214 ± 6 μm) jejunum. In medial jejunum, the ratio of villus height:crypt depth was greater ($P < 0.05$) in CAS than in SPI (2.8 ± 0.1 , 2.4 ± 0.1 , and 2.6 ± 0.1 for CAS, SPI, and SPIA, respectively). The ratio of villus height:crypt depth in distal jejunum was greater ($P < 0.01$) in CAS than in SPI and SPIA (2.6 ± 0.1 , 2.2 ± 0.1 , and 2.2 ± 0.1 for CAS, SPI, and SPIA, respectively). Villus circumferences and crypt depths were greater ($P < 0.05$) in proximal than in medial and distal jejunum. Villus heights and ratios of villus height:crypt depth were lowest ($P < 0.01$) in the distal jejunum.

Intestinal cells labeled with BrdU were found in the crypt region of the intestinal mucosa. The ratio of

BrdU-labeled crypt cells:total crypt cells in proximal (0.28 ± 0.04), medial (0.32 ± 0.03), and distal (0.20 ± 0.02) jejunum was not different among groups, but was lowest ($P < 0.01$) in distal jejunum.

DNA, RNA, and Protein Contents and Enzyme Activities in Jejunal Mucosa

Mucosal RNA and DNA concentrations did not differ among groups (proximal, medial, and distal were 30.7 ± 0.9 and 33.7 ± 1.6 ; 30.7 ± 1.3 and 33.6 ± 1.3 ; and 28.4 ± 1.2 and 29.6 ± 1.9 mg/g of tissue DM, respectively), but concentrations of RNA and DNA were lowest ($P < 0.05$) in the distal jejunum. Mucosal protein concentration in proximal and distal jejunum did not differ among groups (532 ± 23 and 271 ± 31 mg/g of tissue DM for proximal and distal jejunum, respectively), whereas in medial jejunal mucosa, protein concentration was higher ($P < 0.01$) in CAS than in SPI and in SPIA (502 ± 28 , 391 ± 29 , and 350 ± 31 mg/g of tissue DM for CAS, SPI, and SPIA, respectively). Hence, the RNA:protein ratio in medial jejunum tended to be lower ($P < 0.1$) in CAS than in SPI and SPIA (62.1 ± 5.6 , 81.5 ± 7.7 , and 92.9 ± 12.0 for CAS, SPI, and SPIA, respectively). Mucosal protein concentration was higher ($P < 0.01$) and RNA:protein ratio was lower (P

Figure 2. Concentrations of plasma urea before (−30 min) and after (up to +420 min) morning feeding in goats kids fed milk-based diets containing CN (CAS) or soy protein isolate without (SPI) or with AA supplementation (SPIA). Values are means $\pm$ SEM, $n = 8$ per group. Fixed effects (P -value) for urea concentration: time < 0.001 , diet $\times$ time < 0.05 .

< 0.01) in the proximal than in the medial and distal jejunum.

The activities of mucosal APN, DPP-IV, 5'NT, and XO in proximal and distal jejunum did not differ among groups (Table 3). Mucosal activities of APN were higher ($P < 0.01$) in distal than in medial and proximal jejunum. In medial jejunum, activity of mucosal APN tended to be higher ($P < 0.1$) in CAS than in SPI, whereas mucosal DPP-IV activity tended to be lower ($P < 0.1$) in SPI than in SPIA. The activity of mucosal 5'NT was lower ($P < 0.01$) in CAS than in SPI and SPIA, and the activity of mucosal XO was lower ($P < 0.05$) in CAS than in SPI in medial jejunum (Table 3).

Isotopic Enrichments in Blood and Jejunum

^{13}C Enrichments and FSR. The plasma α -[1- ^{13}C] KIC enrichments during the 45-min period after oral administration of L-[1- ^{13}C]leucine increased ($P < 0.001$) but were not affected by dietary treatment and showed no treatment $\times$ time interaction (Figure 4). The cumulative ^{13}C REC in blood CO_2 at 45 min after ingestion of L-[1- ^{13}C]leucine tended to be lower ($P < 0.1$) in CAS than in SPI (Figure 5). The ^{13}C enrichment of protein-bound Leu in mucosa at 45 min after inges-

tion of L-[1- ^{13}C]leucine did not differ among groups in proximal and distal jejunum, but in medial jejunum, ^{13}C enrichment of protein-bound Leu in mucosa was higher ($P < 0.01$) in SPI than in CAS (Table 3). The ^{13}C enrichments were highest ($P < 0.01$) in proximal jejunum. In proximal and distal jejunum, mucosal FSR did not differ among groups, but in medial jejunum, FSR tended to be lower ($P < 0.1$) in CAS than in SPI. Translation (g of protein synthesized/g of RNA per d) was not different after feeding. However, FSR and translational efficiency in mucosa were higher ($P < 0.05$) in proximal than in medial and distal jejunum (Table 4).

^{15}N Enrichments of RNA. The ^{15}N enrichments of mucosal RNA were highest ($P < 0.05$) in proximal jejunum, did not differ among dietary groups in proximal and in distal jejunum, but tended to be higher ($P < 0.1$) in CAS than in SPI in medial jejunum (Table 4).

Correlations

The number of BrdU-labeled crypt cells in jejunum tended to be positively correlated to jejunal crypt depth ($r = 0.22$, $P < 0.1$). In jejunum, mucosal protein con-

Figure 3. Concentrations of plasma total free AA, Thr, Met, and Leu of goat kids fed milk-based diets containing CN (CAS) or soy protein isolate without (SPI) or with AA supplementation (SPIA). Blood samples were collected +45 min relative to the L-[1-¹³C]leucine administration. Values are means $\pm$ SEM, n = 8. ^{a,b}Mean values without a common letter are significantly different. Analysis of variance (*P*-value) total AA: diet = NS, for Thr: diet <0.01, Met: diet <0.05, Leu: diet = NS.

Table 3. Enzyme activities in jejunal mucosa of goat kids fed milk-based diets containing CN or soy protein isolate without or with AA supplementation

Item	Site ¹	Diet ²			SEM ³	<i>P</i> -value ⁴
		CAS	SPI	SPIA		
Amino peptidase N, IU $\times$ 10/mg of protein ⁵	p	274.8	288.1	321.8	27.74	0.5
	m	906.2	641.1	847.3	78.13	0.06
	d	1,199.3	1,150.3	1,109.8	97.80	0.8
Dipeptidyl peptidase IV, IU $\times$ 10/mg of protein ⁵	p	0.08	0.08	0.09	0.01	0.4
	m	0.13	0.10	0.14	0.01	0.1
	d	0.17	0.15	0.21	0.02	0.11
5'-Nucleotidase, IU/mg of protein ⁶	p	57.3	39.9	41.1	7.53	0.3
	m	37.8 ^b	69.6 ^a	60.2 ^a	6.87	0.02
	d	38.7	33.5	32.8	4.93	0.7
Xanthine oxidase, IU/mg of protein ⁷	p	157.1	206.9	254.7	20.93	0.5
	m	189.0 ^b	343.7 ^a	319.7 ^{ab}	34.48	0.02
	d	254.7	280.4	293.4	47.65	0.7

^{a,b}Means within a row with different superscripts differ (*P* < 0.05).

¹Jejunal site: p = proximal; m = medial; d = distal.

²Values are means (n = 8). CAS = milk diet containing acid-precipitated CN (50% of total CP in the diet); SPI = milk diet containing soy protein isolate (50% of total CP in the diet); SPIA = milk diet containing soy protein isolate supplemented with AA (50% of total CP in the diet).

³SEM = pooled standard error of the mean.

⁴Main effect of diet.

⁵IU $\times$ 10 corresponds to the release of 1 nmol of p-nitroanilide per min at 37°C.

⁶One IU corresponds to the release of 1 nmol of phosphate per min at 37°C.

⁷One IU produces 1 nmol uric acid per min at 37°C.

Figure 4. Plasma ketoisocaproic acid (KIC) enrichments ($\alpha[1-^{13}\text{C}]\text{KIC}$) after an oral bolus dose of $\text{L-[1-}^{13}\text{C]leucine}$ in goat kids fed milk-based diets containing CN (CAS) or soy protein isolate without (SPI) or with AA supplementation (SPIA). Values are means $\pm$ SE, $n = 6$ per group. ^{13}C -enrichments are expressed as mole percent excess (MPE). Blood samples were collected +45 min relative to the $\text{L-[1-}^{13}\text{C]leucine}$ administration. Fixed effects (P -value) for ^{13}C KIC: diet = NS, time < 0.001 .

centration was positively correlated to villus circumference ($r = 0.49$, $P < 0.01$) and villus height ($r = 0.44$, $P < 0.01$). In jejunum, FSR was positively correlated to crypt depth ($r = 0.63$, $P < 0.01$) and RNA:protein ratio ($r = 0.55$, $P < 0.05$), and was negatively correlated to villus circumference ($r = -0.56$, $P < 0.05$), villus height ($r = -0.43$, $P < 0.1$), villus height:crypt depth ratio ($r = -0.69$, $P < 0.01$), and mucosal protein concentration ($r = -0.42$, $P = 0.105$).

DISCUSSION

General Aspects

The small intestine of the preruminant lamb is an important organ contributing to 12% of daily whole-body protein synthesis (Attaix and Arnal, 1987). Intestinal protein synthesis responds to the quality of dietary protein, usually assessed by AA composition and protein digestibility. Importantly, soy protein has the capability to inhibit epithelial cell growth, which could have a detrimental effect on mucosal protein accretion caused by changes in the rates of protein synthesis (Deutz et al., 1998). So far, we have investigated the effects of

Figure 5. Recovery of ^{13}C in blood CO_2 after an oral dose of $\text{L-[1-}^{13}\text{C]leucine}$ in goat kids fed milk-based diets containing CN (CAS) or soy protein isolate without (SPI) or with AA supplementation (SPIA). Data are expressed as cumulative recovery over a period of 45 min (means $\pm$ SEM, $n = 6$). Calculation of ^{13}C recovery is based on CO_2 production of $1 \text{ mmol}/(\text{min} \cdot \text{kg BW}^{0.75})$. Analysis of variance (P -value) for ^{13}C recovery: diet < 0.1 .

Table 4. Final ^{13}C enrichment of protein-bound Leu and ^{15}N enrichment of total RNA, fractional protein synthesis rate (FSR) and translational efficiency (k_{RNA}) in jejunal mucosa of goat kids fed milk-based diets containing CN or soy protein isolate without or with AA supplementation

Item	Site ¹	Diet ²			SEM ³	P-value ⁴
		CAS	SPI	SPIA		
^{13}C enrichment of protein-bound Leu, MPE ⁵	p	3.06	3.87	3.53	0.30	0.2
	m	1.43 ^b	2.72 ^a	2.05 ^{ab}	0.25	0.01
	d	1.58	2.06	1.76	0.17	0.17
Fractional protein synthesis rate, %/d	p	240.3	233.5	235.1	20.47	0.9
	m	110.2	163.7	136.6	16.32	0.11
	d	115.5	124.2	117.3	8.62	0.8
Translational efficiency, k_{RNA} ⁶	p	44.2	43.1	39.0	4.21	0.7
	m	17.9	19.4	16.0	2.36	0.6
	d	12.1	10.9	10.6	1.68	0.8
^{15}N enrichment of total RNA, APE ⁷ $\times 10$	p	1.66	1.54	1.23	0.15	0.16
	m	0.92	0.56	0.62	0.22	0.08
	d	0.49	0.65	0.50	0.10	0.5

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Jejunal site: p = proximal; m = medial; d = distal.

²Values are means ($n = 6$). CAS = milk diet containing acid-precipitated CN (50% of total CP in the diet); SPI = milk diet containing soy protein isolate (50% of total CP in the diet); SPIA = milk diet containing soy protein isolate supplemented with AA (50% of total CP in the diet).

³SEM = pooled standard error of the mean.

⁴Main effect of diet.

⁵MPE = mole percent excess.

⁶ $k_{\text{RNA}} = \text{FSR}/100/C_s$, where C_s is total RNA:protein ratio (g of protein synthesized/d per g of RNA).

⁷APE = atom percent excess.

CN replacement by a commercial available soy protein product on jejunal proteins and mucosal RNA synthesis in young goats (Kuhla et al., 2007; Schönhusen et al., 2007, 2010). In the present study, we have used a soy protein isolate and have investigated the effects of soy feeding with and without AA supplement on protein and RNA synthesis in intestinal mucosa.

Growth Performance

The results on growth performance indicate that the partial replacement of CN in a milk-based diet by soy protein isolate both without and with AA supplementation has no effect on BW, ADG, and feed efficiency, which agrees with previous findings (Kuhla et al. 2007; Schönhusen et al., 2010). In studies with 1-mo-old preruminant calves, a higher intake of soy protein than in our study resulted in impaired growth performance if soybean flour, but not soy protein isolate, was fed (Lallès et al., 1995). Reduced growth rate can be attributed to increased amounts of antinutritional factors.

Histomorphometry and Cell Proliferation, Enzyme Activities, and Metabolites

Early studies indicated that within the small intestine, jejunal tissues extract and utilize major proportions of individual dietary AA (Williams, 1969; Ben-Ghedalia et

al., 1974; Tagari and Bergman, 1978). Partial replacement of milk protein by soy protein isolate resulted in alterations of the jejunal structure with reduced villus height and a decreased villus height:crypt depth ratio in the medial and distal part of the jejunum. Therefore, soy protein isolate impaired epithelium growth in mid to distal jejunum, probably leading to a reduced absorptive area in the intestine. On the other hand, crypt depths and BrdU-labeled cells in total jejunum, surprisingly, were not affected by dietary treatment. Previous finding in calves indicated that smaller villi are not necessarily accompanied by lower cell proliferation rates (Sauter et al., 2004), and that villus size can be inversely associated with epithelial cell proliferation rate (Blättler et al., 2001). A greater jejunal villus size in CAS than in SPI could be due to reduced apoptotic rates, greater survival times as observed in preruminant calves by Bittrich et al. (2004), or greater migration rates of epithelial cells from crypts to tips of villi. In addition, the absence of dietary effects on total DNA content in all parts of jejunum indicated that the cell density was not affected. In medial jejunum, decreased villus sizes were accompanied by decreased APN activity. The digestion of proteins and peptides could, therefore, be negatively influenced by soy feeding. The mid to lower jejunum is the area of intensive AA absorption, accounting for 41% of the loss of peptide-linked AA and 57% of the loss of free AA along the small intestine.

tine (Baumrucker and Davis, 1980). In line with this, activities of APN and DPP-IV in the present study were highest in the mid and distal jejunum, supporting findings in neonatal calves (Sauter et al., 2004).

Results from preruminants suggest that food proteins with imbalanced AA patterns such as soy protein inhibit intestinal cell growth and could have a negative effect on protein turnover (Deutz et al., 1998). Our recent morphometric measurements suggest a stimulating effect of AA supplemented to soy protein on small intestinal mucosal growth (Schönhusen et al., 2010). It is, however, important to ascertain that supplementation with AA, known to be at lower concentrations in soy protein isolate than in CN, did not ameliorate soy protein effects on the morphology in a significant manner in the current study. In addition, supplementation of AA to soy protein isolate had a weak effect on the activities of small intestinal brush-border enzymes. In this regard, activity of DPP-IV in mid jejunum tended to be higher in SPIA than in SPI.

RNA and Protein Synthesis

We found a negative correlation between FSR and morphological parameters as well as mucosal protein concentration in jejunum. In preruminant calves fed soy protein concentrate at a level of 20% of total protein in a milk diet, Grant et al. (1989) showed an inverse association between mucosal protein synthesis and mucosal RNA:protein ratio. Moreover, findings of Sève et al. (1986) in young pigs indicate that an increased FSR could be associated with decreased protein deposition in digestive tissues, not affecting the RNA:protein ratio. This is because mucosal protein deposition is the net result of FSR plus proteolysis. We found earlier that soy protein supplemented with IAA resulted in upregulation of jejunal proteins associated with proteolysis (Kuhla et al. 2007).

Mucosal RNA Synthesis. Our data indicated similar mucosal RNA concentrations in SPI and CAS, but lower ^{15}N enrichment in mucosal RNA in SPI than in CAS kids in the mid jejunum. This supports our previous findings that soy protein reduces the ability to salvage preformed nucleic acid precursors of dietary origin for mucosal RNA biosynthesis (Schönhusen et al., 2007, 2010). The excess N-bases and nucleosides were probably oxidized to uric acid (Uauy et al., 1994). In that case, the increased activity of membrane-bound 5'NT in SPI enhanced the availability of nucleosides for absorption, but the higher activity of XO in SPI probably caused a greater degradation of purine bases to uric acid in SPI than in CAS and, therefore, reduced nucleosides for reutilization (Furth-Walker and Amy, 1987). Activities of 5'NT and XO responded to dietary

protein quality, especially to the secretory activity in the brush border; for example, release of anchored glycosyl phosphatidylinositol by hydrolysis (Zimmermann, 1992) and availability of sulfur-containing AA from the diet (Furth-Walker and Amy, 1987), which may affect the balance between de novo synthesis and the reutilization of purine nucleosides by the salvage pathway. High ^{15}N enrichments in mucosal RNA and low specific activities of XO in the proximal jejunum suggest that in the upper jejunum the salvage pathway is pronounced compared with the de novo nucleotide formation (Uauy et al., 1994).

Mucosal Protein Synthesis. Our estimates for FSR along the jejunum were generally higher than values reported for the small intestine of young preruminant lambs (Attaix and Arnal, 1987), growing sheep (Southorn et al., 1992), or growing rats (Krawielitzki et al., 1993), but similar to those reported for growing lambs (Davis et al., 1981). Comparisons among values of intestinal protein synthesis are difficult due to the choice of labeled AA used and differences in the developmental stage of the animals used.

The FSR in the medial jejunum tended to be increased in the SPI group compared with the CAS group, and AA supplementation to soy protein isolate (SPIA) did not overcome the soy effects on FSR. As a result, SPIA kids had a similar mucosal protein concentration and translational capacity in the mid jejunum as SPI kids, which suggests that free dietary AA are not utilized in the same way and at the same efficiency as protein-bound AA for mucosal protein synthesis. The measured FSR of 164%/d means that the mid-jejunal mucosa in SPI is synthesizing protein at a rate 50% faster than in CAS, and 27% faster than in SPIA. This could be due mainly to accelerated cell or protein turnover (McNurlan et al., 1979). This is supported by the positive correlation of FSR with crypt depth and RNA:protein ratio, whereas villus size was negatively correlated to FSR. These findings support the negative relationship between villus size and cell proliferative activity in mucosa cells. The negative association of FSR with mucosal protein concentration suggests an increased cell turnover and a significant loss of protein in the jejunal mucosa. The ^{13}C REC in blood CO_2 tended to be higher when SPI was fed compared with CAS suggesting higher leucine oxidation. In line with this is the finding of a higher L-[1- ^{13}C]leucine incorporation in mucosal protein at the mid-jejunum and can be interpreted to mean an increased protein turnover reflecting a repair response after feeding SPI.

Supplementation of AA to soy protein isolate reduced Leu oxidation and diminished the incorporation of Leu into mucosa protein as well as the FSR in jejunum in SPIA, but showed no clear changes compared with

SPI. Nieto et al. (1994) showed that supplementation of Met and Lys to a soybean diet in growing chicken did not change the FSR in the jejunum compared with a soybean diet without supplementation. Metges et al. (2000) reported that free leucine raises plasma and tissue AA concentrations, and leads to higher oxidation and lower whole-body net protein synthesis. We found that feeding free AA together with soy protein-bound AA in SPIA did not result in a higher plasma and tissue total protein concentration, and plasma total protein concentration was at the same level as after SPI feeding, but consistently lower than in CAS. Plasma urea concentrations as an indication for protein degradation showed no changes in SPIA compared with SPI, but in contrast to CAS, urea concentrations tended to decrease after feed intake in SPI and SPIA. Results obtained in pigs fed CN versus soy protein indicate that AA uptake by the gut tended to increase, whereas portal flux, liver uptake, and urea production were reduced (Deutz et al., 1998).

Despite possible differences in the pattern of absorbed AA entering the portal circulation after soy protein or CN feeding (Metges et al., 2000; Daenzer et al., 2001), the total concentration of dietary AA appearing in blood plasma of goat kids did not differ between SPI and CAS, but lower plasma concentrations of Thr and Met were found in SPI kids compared with CAS kids. This agrees with observations in pigs consuming a diet containing soy protein isolate instead of CN (Barth et al., 1990). In calves sensitized against soy protein, the appearance of 3-methylhistidine in blood plasma, used as a marker for AA absorption, was not changed compared with calves that were not sensitized (Vacher et al., 1990). We found that supplementation of AA to soy protein (SPIA) did not result in higher plasma concentrations of total AA and Thr but did increase the plasma concentration of Met. It is likely that supplemented Thr was used for intestinal protein synthesis to maintain an adequate amount of mucins and proteins for cellular defense in mucosa (Schaart et al., 2005).

The differences in FSR between the proximal and distal jejunum were largely due to differences in the [$1\text{-}^{13}\text{C}$]leucine enrichments of the mucosa protein-bound fraction. In general, the ^{13}C enrichments were highest in proximal jejunum; moreover, the decrease of FSR between the proximal and distal jejunum was accompanied by an increased mucosal APN activity. This suggests that the luminal availability and mucosal uptake of labeled enteral leucine was progressively diminished toward the terminal jejunum. Highest values for FSR in the proximal jejunum were also observed in neonatal (Stoll et al., 2000) and growing pigs (Bregendahl et al., 2004) and growing lambs (Abdul-Razzaq and Bickerstaffe, 1989).

CONCLUSIONS

Milk replacer formulas with alternative protein sources for suckling preruminants containing a plant protein source, especially soy protein, have more economic relevance to breeding. Our study provides information on the applicability of soy protein in milk replacer diets for preruminants to retain breeding success of the animals. We showed that soy protein isolate as partial replacement of CN in milk diets changes the mucosal protein synthesis in the jejunum of young goats, but we could not demonstrate that supplementation of IAA to soy protein isolate ameliorates this soy effect. Morphological changes in the jejunal mucosa induced by feeding of soy protein isolate are accompanied by reduced protein content, less incorporation of preformed dietary RNA precursors into RNA, and a trend for increased protein synthesis in intestinal mucosa. The trend for an increased protein synthesis and the tendency for a higher Leu oxidation are an indication of increased protein turnover in the soy group, but may not reflect improved intestinal function and point to enhanced repair processes in enterocytes. Soy protein supplemented with free AA does not ameliorate soy protein effects on intestinal mucosa growth in a significant manner, but does counteract some soy feeding effects on protein utilization in the mucosa and blood. Balancing the AA composition of soy protein by supplementation of IAA in a free form barely improves intestinal morphology and function and does not ameliorate the protein quality of soy protein in milk replacer diets.

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REFERENCES

- Abdul-Razzaq, H. A., and R. Bickerstaffe. 1989. The influence of rumen volatile fatty acids on protein metabolism in growing lambs. *Br. J. Nutr.* 62:297-310.
- Attaix, D., and M. Arnal. 1987. Protein synthesis and growth in the gastrointestinal tract of the young preruminant lamb. *Br. J. Nutr.* 58:159-169.
- Barth, C., K. E. Scholz-Ahrens, M. de Vrese, and A. Hotze. 1990. Difference in Plasma amino acids following casein or soy protein intake: Significance for differences of serum lipide concentrations. *J. Nutr. Sci. Vitaminol. (Tokyo)* 36:S111-S117.
- Baumrucker, C. R., and C. L. Davis. 1980. Glutamyltranspeptidase activity along the small intestine of sheep: potential areas of amino acids and peptide transport. *J. Dairy Sci.* 63:379-384.
- Ben-Ghedalia, D., H. Tagari, and A. Bondi. 1974. Protein digestion in the intestine of sheep. *Br. J. Nutr.* 33:125-142.

- Bezabih, M., and E. Pfeffer. 2003. Body chemical composition and efficiency of energy and nutrient utilization by growing pre-ruminant Saanen goat kids. *J. Anim. Sci.* 77:155–163.
- Bittrich, S., C. Philipona, H. M. Hammon, V. Rom, P. Guilloteau, and J. W. Blum. 2004. Preterm as compared with full-term calves are characterized by morphological and functional immaturity of the small intestine. *J. Dairy Sci.* 87:1786–1795.
- Blättler, U., H. M. Hammon, C. Morel, C. Philipona, A. Rauprich, V. Rome, I. LeHuerou-Luron, P. Guilloteau, and J. W. Blum. 2001. Feeding colostrum, its composition and feeding duration variably modify proliferation and morphology of the intestine and digestive enzyme activities of neonatal calves. *J. Dairy Sci.* 84:1256–1263.
- Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
- Bregendahl, K., L. Lui, J. P. Cant, H. S. Bayley, B. W. McBride, L. P. Milligan, J.-J. Yen, and M. Z. Fan. 2004. Fractional protein synthesis rates measured by intraperitoneal injection of a flooding dose of L-[ring-²H₂]phenylalanine in pigs. *J. Nutr.* 134:2722–2728.
- Burrin, D. G., T. A. Davis, S. Ebner, P. A. Schoknecht, M. L. Fiorotto, and P. Reeds. 1997. Colostrum enhances the nutritional stimulation of vital protein synthesis in neonatal pigs. *J. Nutr.* 127:1284–1289.
- Burrin, D. G., T. A. Davis, M. L. Fiorotto, and P. Reeds. 1991. Stage development and fasting affect protein synthetic activity in the gastrointestinal tissue of suckling rats. *J. Nutr.* 121:1099–1108.
- Daenzer, M., K. J. Petzke, B. J. Bequette, and C. C. Metges. 2001. Whole-body nitrogen and splanchnic amino acid metabolism differs in rats fed mixed diets containing casein or its corresponding amino acid mixture. *J. Nutr.* 131:1965–1972.
- Davis, S. R., T. N. Barry, and G. A. Hughson. 1981. Protein synthesis in tissue of growing lambs. *Br. J. Nutr.* 46:409–419.
- Degen, G. H., P. Janning, P. Diel, and H. M. Bolt. 2002. Estrogenic isoflavones in rodent diets. *Toxicol. Lett.* 128:145–157.
- Deutz, N. E. P., M. J. Bruins, and P. B. Soeters. 1998. Infusion of soy and casein protein meals affects interorgan amino acid metabolism and urea kinetics differently in pigs. *J. Nutr.* 128:2435–2445.
- Faure, M., D. Moënnaz, F. Montignon, L. B. Fay, D. Breuillé, P. A. Finot, O. Ballèvre, and J. Boza. 2002. Development of a rapid and convenient method to purify mucins and determine their *in vivo* synthesis rate in rats. *Anal. Biochem.* 307:224–251.
- Faure, M., D. Moënnaz, F. Montignon, C. Mettraux, D. Breuillé, and O. Ballèvre. 2005. Dietary threonine restriction specifically reduces intestinal mucin synthesis in rats. *J. Nutr.* 135:486–491.
- Furth-Walker, D., and N. K. Amy. 1987. Regulation of xanthine oxidase activity and immunological detectable protein in rats in response to dietary protein and iron. *J. Nutr.* 117:1697–1703.
- Garlick, P. J., and A. M. McNurlan. 1998. Measurement of protein synthesis in human tissues by the flooding method. *Curr. Opin. Clin. Nutr. Metab. Care* 1:455–460.
- Grant, A. L., R. E. Holland, J. W. Thomas, K. J. King, and J. S. Liesman. 1989. Effects of dietary amines on the small intestine in calves fed soybean protein. *J. Nutr.* 119:1034–1041.
- Hennig, U., C. C. Metges, A. Berk, A. Tuchscherer, and M. Kwella. 2004. Relative ileal amino acid flows and microbial counts in intestinal effluents of Goettingen Minipigs and Saddleback pigs are not different. *J. Anim. Sci.* 82:1976–1985.
- Ipata, P. L. 1967. A coupled optical enzyme assay for 5'-nucleotidase. *Anal. Biochem.* 20:30–36.
- Jentsch, W., A. Chudy, and M. Beyer. 2003. Reference numbers of feed value and requirement on the base of net energy. Rostock Feed Evaluation System. Plexus Verlag, Miltenberg-Frankfurt, Germany.
- Junghans, P., J. Voigt, W. Jentsch, C. C. Metges, and M. Derno. 2007. The ¹³C bicarbonate dilution technique to determine energy expenditure in young bulls validated by indirect calorimetry. *Livest. Sci.* 110:280–287.
- Kanjanapruthipong, J. 1998. Supplementation of milk replacers containing soy protein with threonine, methionine, and lysine in the diets of calves. *J. Dairy Sci.* 81:2912–2915.
- Kien, C. L. 1989. Isotopic dilution of CO₂ as an estimate of CO₂ production during substrate oxidation studies. *Am. J. Physiol.* 257:E296–E298.
- Krawielitzki, K., R. Schadereit, and F. Kreienbring. 1993. Application of ¹⁵N-labeled lysine for the estimation of fractional protein synthesis rates by the flooding method. *Arch. Anim. Nutr.* 43:303–312.
- Kuhla, S., P. E. Rudolph, D. Albrecht, U. Schönhusen, R. Zitnan, W. Tomek, K. Huber, J. Voigt, and C. C. Metges. 2007. A milk diet partly containing soy protein does not change growth but regulates jejunal proteins in young goats. *J. Dairy Sci.* 90:4334–4345.
- Lallès, J. P., R. Toullec, P. Branco Parda, and J. W. Sissons. 1995. Hydrolyzed soy protein isolate sustains high nutritional performance in veal calves. *J. Dairy Sci.* 78:194–204.
- Lobley, G. E., A. Connell, E. Milne, A. M. Newman, and T. A. Ewing. 1994. Protein synthesis in splanchnic tissues of sheep offered two levels of intake. *Br. J. Nutr.* 71:3–12.
- Maroux, S., M. Louvard, and J. Baratti. 1973. The aminopeptidase from intestinal brush border. *Biochim. Biophys. Acta* 321:282–295.
- Matsuura, S., and K. Suzuki. 1997. Immunohistochemical analysis of DNA synthesis during chronic stimulation with isoproterenol in mouse submandibular gland. *J. Histochem. Cytochem.* 45:1137–1145.
- McNurlan, M. A., A. M. Tomkins, and P. J. Garlick. 1979. The effect of starvation on the rate of protein synthesis in the rat liver and small intestine. *Biochem. J.* 178:373–379.
- Metges, C. C., and M. Daenzer. 2000. ¹³C gas chromatography-combustion isotope ratio mass spectrometry analysis of *N*-pivaloyl amino esters of tissue and plasma samples. *Anal. Biochem.* 278:156–164.
- Metges, C. C., A. E. El-Khoury, A. B. Selvaraj, R. H. Tay, A. Atkinson, M. M. Regan, B. J. Bequette, and V. R. Young. 2000. Kinetics of L-[1–¹³]leucine when ingested with free amino acids, unlabelled or intrinsically labeled casein. *Am. J. Physiol. Endocrinol. Metab.* 278:E1000–E1009.
- Montagne, L., R. Toullec, and J. P. Lallès. 2001. Intestinal digestion of dietary and endogenous proteins along the small intestine of calves fed soybean or potato. *J. Anim. Sci.* 79:2719–2730.
- Montagne, L., R. Toullec, T. Savidge, and L. P. Lallès. 1999. Morphology and enzyme activities of the small intestine are modulated by the dietary protein source in the pre-ruminant calf. *Reprod. Nutr. Dev.* 39:455–466.
- Nagatsu, T., M. Hino, H. Fuyamada, T. Hayakawa, S. Sakakibara, Y. Nakagawa, and T. Takemoto. 1976. New chromogenic substrates for X-propyl-di-peptidyl-aminopeptidase. *Anal. Biochem.* 74:466–476.
- Naumann, C., and R. Bassler. 1993. Chemical Analysis of Feeds: Books of Methods. Vol. 3. VDLUFA-Verlag, Darmstadt, Germany.
- Nieto, R., R. M. Palmer, I. Fernández-Figares, L. Péres, and C. Prieto. 1994. Effect of dietary protein quality, feed restriction, and short-term fasting on protein synthesis and turnover in tissues of the growing chicken. *Br. J. Nutr.* 72:499–507.
- Rufibach, K., N. Stefanoni, V. Rey-Roethlisberger, P. Schreiner, M. G. Doherr, L. Tappy, and J. W. Blum. 2006. Protein synthesis in jejunum and liver of neonatal calves fed vitamin A and lactoferrin. *J. Dairy Sci.* 89:3075–3086.
- SAS Institute. 2004. SAS User's Guide: Statistics. Version 9.1 ed. SAS Inst. Inc., Cary, NC.
- Sauter, S. N., B. Roffler, C. Philipona, C. Morel, V. Rom, P. Guilloteau, J. W. Blum, and H. M. Hammon. 2004. Intestinal development in neonatal calves. Effects of glucocorticoids and dependence on colostrum feeding. *Biol. Neonate* 85:94–104.
- Schaart, M. W., H. Schierbeek, S. R. D. van der Schoor, B. Stoll, D. G. Burrin, P. Reeds, and J. B. van Goudoever. 2005. Threonine utilization is high in the intestine of piglets. *J. Nutr.* 135:766–770.
- Schönhusen, U., S. Kuhla, P. E. Rudolph, R. Zitnan, D. Albrecht, K. Huber, J. Voigt, A. Flöter, H. M. Hammon, and C. C. Metges. 2010. Alterations in the jejunum of young goats caused by feeding soy protein-based diets. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 94:1–4.

- Schönhusen, U., S. Kuhla, R. Zitnan, K. D. Wutzke, K. Huber, S. Moors, and J. Voigt. 2007. Effect of a soy protein-based diet on ribonucleic acid metabolism in the small intestinal mucosa of goat kids. *J. Dairy Sci.* 90:2404–2412.
- Seegraber, F. J., and J. L. Morill. 1986. Effect of protein on calves' intestinal absorptive ability and morphology determined by scanning electron microscopy. *J. Dairy Sci.* 65:1962–1970.
- Sève, B., P. J. Reeds, M. F. Fuller, A. Cadenhead, and S. M. Hay. 1986. Protein synthesis and retention in some tissues of the young pig as influenced by dietary protein intake after early-weaning. Possible connection to the energy metabolism. *Reprod. Nutr. Dev.* 26:849–861.
- Southorn, B., J. M. Kelly, and B. W. McBride. 1992. Phenylalanine flooding dose procedure is effective in measuring the intestinal and liver protein synthesis in sheep. *J. Nutr.* 122:2398–2407.
- Stoll, B., X. Chang, M. Z. Fan, P. Reeds, and D. G. Burrin. 2000. Enteral nutrient intake level determines intestinal protein synthesis and accretion rates in neonatal pigs. *Am. J. Physiol.* 279:G288–G294.
- Stoll, B., J. Henry, P. J. Reeds, H. Yu, F. Jahoor, and D. G. Burrin. 1998. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. *J. Nutr.* 128:606–614.
- Tagari, H., and E. N. Bergman. 1978. Intestinal disappearance and portal blood appearance of amino acids in sheep. *J. Nutr.* 108:790–803.
- Uauy, R., R. Quan, and A. Gil. 1994. Role of nucleotides in intestinal development and Repair: Implications for infant nutrition. *J. Nutr.* 124:1436S–1441S.
- Vacher, P.-Y., M. Schmitz, H. Hirni, and J. W. Blum. 1990. Concentration plasmatique postprandiale de 3-méthylhistidine comparée à celle de lysine, d'homoarginine et de xylose dans des conditions normales et en cas de malabsorption chez le veau préruminant. *Reprod. Nutr. Dev.* 30:471–482.
- Wang, X., Y. Yin, L. Yue, Z. Wang, and G. Wu. 2007. A deficiency or excess of dietary threonine reduces protein synthesis in jejunum and skeletal muscle of young pigs. *J. Nutr.* 137:1442–1446.
- Williams, V. J. 1969. The relative rates of absorption of amino acids from the small intestines of the sheep. *Comp. Biochem. Physiol.* 29:865–870.
- Zimmermann, H. 1992. 5'-Nucleotidase: Molecular structure and functional aspects. *Biochem. J.* 285:345–365.