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A Milk Diet Partly Containing Soy Protein Does Not Change Growth but Regulates Jejunal Proteins in Young Goats[1]

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ABSTRACT

Soy protein is known to alter intestinal function and structure. We determined in young goats whether a diet partly containing soy protein differently affects intestinal morphology and the jejunal and hepatic proteome as compared with a milk diet. Fourteen male 2-wk-old White German dairy goat kids were fed comparable diets based on whole cow's milk in which 35% of the crude protein was casein (milk protein group; MP) or soy protein supplemented by indispensable AA (SPAA) for 34 d (n = 7/group). Body weight gain and food efficiency were not different. Jejunal and hepatic tissue was collected to determine intestinal morphology by microscopy and protein repertoire by 2-dimensional gel electrophoresis and mass spectrometry. Jejunal crypt depth was reduced and villus height to crypt depth ratio was higher in SPAA than in milk protein. Out of 131 proteins identified, 32 proteins were found to be differently expressed in both groups. In SPAA, downregulated jejunal proteins were involved in processes related to cytoskeleton generation, protein, lipid, and energy metabolism. Downregulated hepatic proteins were related to glycolysis and Krebs cycle. Thirteen proteins were upregulated in SPAA. Among these, 2 hepatic proteins were related to carbohydrate breakdown. The other 11 jejunal proteins were involved in cytoskeleton assembly, proteolysis, and carbohydrate breakdown. In addition, glutathione-S-transferase was found to be upregulated in the medial jejunum. In conclusion, a SPAA diet as compared with a milk diet was related to changes in jejunal morphology and jejunal proteins relevant for protein turnover, energy metabolism, and cytoskeleton assembly with no apparent impact on animal BW gain.
Key words: intestinal development, goat kid, proteome analysis, soy protein

INTRODUCTION

Alternate-protein milk replacer formulas for suckling preruminants containing 50% or less of total protein as soy protein (**SP**) have been shown to reduce growth and feed efficiency in young ruminants when compared with whole milk or casein diets (Lallès, 1993; Kanjanapruthipong, 1998; Drackley et al., 2006). This might be associated to changes of small intestinal mucosa with deterioration of villus integrity and decreased villus height and crypt cell depth (Seegraber and Morrill, 1986; Zitnan et al., 2005; Drackley et al., 2006) with consequences for absorptive function (Seegraber and Morrill, 1986; Kanjanapruthipong, 1998). In line with this are findings showing SP to support growth and gut and liver protein synthesis in rats and pigs to a lesser degree than casein meals (Deutz et al., 1998; Tachibana et al., 2005). A diet containing SP as the sole protein source alters hepatic gene and protein expression in young pigs (Schwerin et al., 2002; Junghans et al., 2004b). Besides isoflavones and antigenic factors such as beta-conglycinine present in SP (Payne et al., 2001; Ren et al., 2001), a known deficit of indispensable AA can be responsible for the above-mentioned effects, and supplementation of dietary SP with indispensable AA (Lys, Met, Thr) improved growth and nitrogen accretion in young calves (Pelaez and Walker, 1979; Kanjanapruthipong, 1998). We recently found an altered RNA metabolism in the small intestinal mucosa of young goats fed a diet partly containing SP supplemented with AA as compared with a whole milk/casein-based diet (Schoenhusen et al., 2007). We therefore were interested to explore whether this diet also changes protein expression patterns in

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Table 1. Ingredients and chemical composition of milk or soy protein containing diets fed to goat kids

	Diet[1]	
	MP	SPAA
Ingredient, g/kg of DM feed		
Cow milk	752	727
Casein[2]	100	—
Soy protein product[3]	—	160
Lactose	148	104
AA mixture[4]	—	9
Chemical composition		
DM, g/kg	170	169
Crude ash, g/kg of DM	41	49
CP, g/kg of DM	307	307
Ether extract, g/kg of DM	242	240
Crude fiber, g/kg of DM	0	6
Nitrogen-free extract, g/kg of DM	410	398
Lactose, g/kg of DM	403	351
ME, MJ/kg of DM	17.8	17.5

[1]MP = milk diet containing CN (35% of the milk CP was replaced by casein). SPAA = milk diet containing soy protein product supplemented with AA (35% of total CP in the diet).

[2]Acid-precipitated casein, Molkereigesellschaft Lauingen GmbH, Lauingen, Germany.

[3]Soy product Hamlet 300, Hamlet Protein A/S, Horsens, DK. Composition of the soy protein product (g per 100 g DM): crude ash, 6.3, CP, 61.8, ether extract, 1.8, crude fiber, 3.8. Isoflavone content (per kg of DM soy protein product): 907 mg of daidzein, 1,959 mg of genistein.

[4]Composition (g per 100 g of AA mixture): Thr, 5; Val, 19; Ile, 7; Leu, 24; His, 6; Lys, 23; Met, 16.

Table 2. Crude protein (g/100 g of DM) and AA contents (g/16 g of N) of milk or soy protein containing diets

	Diet[1]	
Item	MP	SPAA
CP	30.69	30.73
Asp	6.84	7.78
Thr	4.02	3.96
Ser	5.00	4.64
Glu	21.28	19.03
Gly	1.78	2.43
Ala	3.06	3.46
Val	5.93	6.00
Ile	5.02	4.98
Leu	9.03	9.06
Tyr	3.91	3.28
Phe	4.78	4.76
His	2.67	2.56
Lys	7.87	7.74
Arg	3.26	4.10
Pro	9.74	7.73
Cys	0.65	0.96
Met	2.30	2.20
Trp	1.31	1.26

[1]MP = milk diet containing CN (35% of the milk CP was replaced by casein). SPAA = milk diet containing soy protein product supplemented with AA (Thr, Val, Ile, Leu, His, Lys, Met; 35% of total CP in the diet).

jejunal and liver protein, bearing in mind that specific proteins must be involved in the processes leading to the above-described structural and functional alterations.

MATERIALS AND METHODS

Animals, Feeding, and Experimental Procedures

Goat kids were housed and treated in accordance with the guidelines for the use of animals as experimental subjects of the state government in Mecklenburg-West Pommerania. Fourteen male kids (White German dairy goat) were purchased from a commercial goat farm at 6 d of age. After birth the kids stayed with their mothers to suck colostrum and milk. Upon arrival at the research institute, mean BW was 4.5 ± 0.2 kg and the kids were fed cow's milk by nipple bottle 3 times a day (0700, 1200, and 1600 h). Goat kids were then randomly assigned to 2 dietary treatment groups of 7 kids each. Groups were housed in boxes (3 m × 2.5 m) at an ambient temperature of about 15°C with free access to fresh water. At 14 d of age feeding of experimental diets was started and continued for 34 d. Both experimental diets (17% DM, 5.2% protein, 4.1% fat; Table 1) were based on whole cow's milk (13% DM, 3.3% protein, 3.6% fat, 4.9% lactose). In the milk group (**MP**) 35% of the milk protein was replaced by pure acid-precipitated casein (Molkereigenossenschaft GmbH, Lauingen, Germany), whereas in the soy protein group the same amount of milk protein was replaced by SP (Soy protein product HP 300, Hamlet Protein A/S, Horsens, Denmark; 6.3% crude ash, 61.8% CP, 1.8% ether extract, 3.8% crude fiber in DM) supplemented by those AA known to be lower concentrated in soy protein than in casein (Thr, Val, Ile, Leu, His, Lys, Met; **SPAA**; Tables 1 and 2). Diets were formulated to be isonitrogenous and isocaloric (Tables 1 and 2). Lactose was added to provide a constant protein to N-free extract ratio in both diets.

Preparation of experimental diets and feeding level were as described by Schoenhusen et al. (2007). Two kids had to be removed from the MP group because of clinical pneumonia.

On d 34, 5 h after the morning feeding, kids were killed by stunning using a captive bolt pistol and bled from the carotid arteries. Blood samples were taken in tubes containing di-K-EDTA (1.6 mg/mL of blood) and held on crushed ice. After centrifugation (1,500 × *g*, 15 min at 4°C) plasma aliquots were stored at –20°C for later analysis. Liver and segments from the proximal and medial jejunum were removed within 5 min after slaughter. The segments were rinsed free of digesta with ice-cold saline [0.9% (wt/vol) NaCl]. Tissue sections of 1 cm^2 from each jejunal segment were cut and fixed in 4% formaldehyde solution for evaluation of jejunal mucosa morphology. Mucosal tissue was harvested