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Effects of dietary leucine and phenylalanine on pancreas development, enzyme activity, and relative gene expression in milk-fed Holstein dairy calves

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ABSTRACT

This study aimed to investigate the effect of dietary supplementation with leucine and phenylalanine on pancreas development, enzyme activity, and related gene expression in male Holstein calves. Twenty male Holstein calves [1 d of age, 38 ± 3 kg of body weight (BW)] were randomly assigned to 1 of the following 4 treatment groups with 5 calves in each group: control, leucine supplementation (1.435 g/L of milk), phenylalanine supplementation (0.725 g/L of milk), and leucine and phenylalanine (1.435 + 0.725 g/L of milk). The diets were made isonitrogenous with the inclusion of alanine in each respective treatment. The feeding trial lasted for 8 wk, including 1 wk for adaption and 7 wk for the feeding experiment. Leucine tended to increase the concentration of total pancreatic protein (mg/kg of BW). Phenylalanine increased the concentrations of plasma insulin, cholecystokinin, and pancreatic DNA (mg/g) and the expression of trypsin gene but decreased the pancreatic protein:DNA ratio and tended to decrease the pancreas weight (g/kg of BW). No differences were observed in total pancreatic DNA (mg/pancreas and mg/kg of BW), pancreatic protein (mg/pancreas), or activities of α-amylase, trypsin, and lipase. The relative expression levels of the genes encoding α-amylase and lipase did not differ among the 4 groups. The supplementation of both leucine and phenylalanine showed an interaction on the pancreas weight (g and g/kg of BW) and a tendency of an interaction on the pancreatic protein concentration (mg/g of pancreas and mg/kg of BW) and the plasma glucose concentration. Leucine tended to increase the size of the pancreatic cells, whereas phenylalanine tended to increase the number of pancreatic cells. However, neither AA affected the activities of the pancreatic enzymes of the calves. These results indicate that leucine and phenylalanine supplementation in milk-fed Holstein calves differentially affect pancreatic growth and development.
Key words: calf, leucine, pancreas, phenylalanine

INTRODUCTION

The composition and content of dietary carbohydrates are key factors affecting rumen fermentation and the health and productivity of ruminants. To achieve the full genetic potential of lactating cows, diets rich in starch could be fed to increase milk production (Herrera-Saldana et al., 1990). The starch is first degraded in the rumen by amylolytic microbes to produce VFA, predominantly acetate, propionate, and butyrate, which are absorbed into the rumen wall. In ruminants, starch that resists microbial enzymatic degradation in the upper gut sections (i.e., forestomachs) may be termed bypass starch, undegradable starch, or ruminally resistant starch. More precisely, ruminally resistant starch is expected to resist degradation mechanisms in the rumen and be digested in the small intestine (Deckardt et al., 2013). About 18 to 42% of the starch bypasses rumen degradation and enters the small intestine (Owens et al., 1986), which could supply about 30% of total glucose needs (Huntington, 1997). Pancreatic α-amylase mediates the hydrolysis of rumen bypass starch to oligosaccharides or membrane-bound intestinal α-glucosidases, which hydrolyze these oligosaccharides to glucose in the small intestines. The released glucose is absorbed by the intestinal epithelial cells, providing the most important source of exogenous glucose for ruminants. The energy efficiency of starch in the small intestine is 42% higher than that in the rumen (Huntington et al., 2006). However, the digestibility of the small intestine for starch in dairy cows is on average 35 to 60% (Harmon et al., 2004), and thus some of the rumen bypass starch may not be digested, resulting in energy used inefficiently and health risks (Gressley et al., 2011). Zinn et al. (2002) revealed that the increase in digestibility depends on disruption of the protective protein matrix surrounding the starch

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granules rather than simply increasing starch solubility. Several studies have shown that insufficient secretion of pancreatic α-amylase is the limiting factor in the utilization of rumen bypass starch (Swanson et al., 2002; Harmon et al., 2004). Therefore, increasing the secretion of pancreatic α-amylase in dairy cows could be an approach to improve the energy supply and production performance of high-yielding dairy cows.

The pancreas is an important gland in the animal digestive system. Internal and external pancreatic secretions are essential for nutrient digestion and the regulation of several physiological processes. The pancreatic juice secreted by the pancreas is rich in bicarbonate and contains several types of digestive enzymes, including trypsin, pancreatic lipase, and pancreatic amylase, all of which are important hydrolases and play important roles in digestion and absorption (Rinderknecht et al., 1983). The secretion of pancreatic juice is affected by many factors, such as the age of the animal (Jiang et al., 2013), insulin (Baile et al., 1969), cholecystokinin (Owyang, 1994), dietary protein (Hara et al., 1996), and energy levels (de Dios et al., 1988). The effects of dietary protein and AA on the synthesis and secretion of trypsin have attracted much research interests. Richards et al. (2002) showed that in growing steers (initial BW = 379 kg) infused with starch (1,042 g/d) into the small intestine, the concentration of pancreatic α-amylase linearly increased in a dose-responsive manner to the amounts of infused casein (0, 50, 100, 150, and 200 g/d). Greater quantities of starch disappeared with increased casein infusion, and the infusion of 200 g/d of casein increased small intestinal starch disappearance by 226 g/d over the control. Swanson et al. (2008) obtained similar results by increasing the rumen bypass of soybean meal in yearling beef steers (initial BW = 510 kg). The studies of Liao et al. (2009) in Angus steers (initial BW = 260 kg) and Swanson et al. (2004) in Angus steers (initial BW = 290 kg) also revealed a positive dynamic relationship between the supply of protein and the secretion of pancreatic amylase. These authors also observed that a change in the ratio of the EAA to starch in the small intestine could change pancreatic amylase secretion. By infusing casein, EAA, NEAA, and glutamate into the duodenum, Brake et al. (2014) found that casein and EAA increased the secretion of pancreatic amylase without affecting the starch digestibility in the small intestine, whereas NEAA and glutamate increased the starch digestibility (steer initial BW = 259 kg). Yu et al. (2014) showed that both protein and AA could influence the activities of pancreatic enzymes and the expression of their genes in yearling ewes (initial BW = 297 kg) by altering pancreas development.

Amino acids traditionally were classified as nutritionally essential or nonessential for animals and humans based on nitrogen balance and growth (Wu, 2010). A key element of this classification is that all NEAA are assumed to be synthesized adequately in the body to meet the needs for protein synthesis (Wu, 2013). Therefore, Wu (2010) proposed a concept of functional AA, which is defined as those AA that participate in and regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of the organisms. Leucine can regulate protein synthesis and catabolism in animals by activating the mammalian target of rapamycin pathway (Boultwood et al., 2013; Kim et al., 2013). Phenylalanine is an aromatic AA that can promote the secretion of cholecystokinin (**CCK**) through the calcium-sensing receptors (Liou et al., 2011). Cholecystokinin can promote the synthesis of pancreatic amylase, trypsin, and trypsinogen, stimulate the release of these pancreatic enzymes, and enhance their activities. Duodenal infusions of phenylalanine (Yu et al., 2013) and leucine (Liu et al., 2015) play a role in regulating the secretion of pancreatic α-amylase.

The above studies showed that AA play a regulatory role in pancreas secretion and the gene expression of pancreatic enzymes at both transcriptional and translational levels. We thus hypothesized that supplementation of leucine or phenylalanine further strengthens their roles in the regulation of pancreatic metabolism, leading to an increment of exogenous enzyme production. We noted that the aforementioned studies used cannulated animals to infuse the substrates via the cannula to avoid ruminal degradation, and influences of the surgery preparation on the results remain undefined. Therefore, the present study investigated the effects of supplemental leucine and phenylalanine on pancreas development, enzymatic activity, and transcriptional regulation in milk-fed Holstein dairy calves without any surgical preparation.

MATERIALS AND METHODS

The use of the animals and experimental protocols was approved by the Animal Care and Use Committee of the College of Animal Science and Technology, the Northwest A&F University (Yangling, Shaanxi, China).

Animals, Experimental Design, and Diets

Twenty male Holstein calves (1 d of age, 38 ± 3 kg of BW) were used in this study. Within 1 h after birth, each calf was fed 4.0 L of colostrum with an IgG concentration >50 g/L. The calves were randomly assigned