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Influence of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activity of lamb offspring

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Abstract

To determine the influence of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activity in lambs, 31 multiparous, Rambouillet ewes were allocated to one of three dietary treatments at 54 d of gestation. Dietary treatments were 100% of nutrient requirements (control, CON; n = 11), 60% of control (restricted, RES; n = 10), or RES plus 180 mg rumen-protected arginine•kg BW⁻¹•d⁻¹ (RES-ARG; n = 10). Immediately after parturition, lambs were removed from dams and reared independently. Milk-replacer and alfalfa hay + creep feed were offered for *ad libitum* intake. At day 54 of age, lambs were slaughtered and the pancreas and small intestine were collected. Pancreatic (α -amylase and trypsin) and jejunal (maltase, glucoamylase, sucrase, isomaltase, and lactase) digestive enzyme activities were assayed. Data were analyzed using the GLM procedure of SAS for effects of treatment. Contrast statements were used to determine differences between means for effects of restriction (CON vs. RES and RES-ARG) and rumen-protected arginine supplementation (RES vs. RES-ARG). There was no influence ($P = 0.15$) of maternal nutrient restriction or rumen-protected arginine supplementation on pancreatic or jejunal protein concentrations. No treatment effects were observed ($P = 0.12$) for enzymes involved in starch digestion including pancreatic α -amylase and jejunal maltase, glucoamylase, and isomaltase. Sucrase activity was undetected in the jejunum of lambs across all treatments. Maternal nutrient restriction tended to increase ($P = 0.08$) pancreatic trypsin activity per gram protein in lambs. Lactase activity per gram protein in the jejunum of lambs tended to decrease ($P = 0.09$) with maternal nutrient restriction. Rumen-protected arginine

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Declaration of interest

Declarations of interest: none.

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supplementation to gestating ewes did not influence ($P = 0.19$) digestive enzyme activities of lamb offspring. These data suggest that maternal nutrient restriction and rumen-protected arginine supplementation have minimal effects on digestive enzyme activity in offspring.

Keywords

developmental programming; digestive enzymes; maternal nutrition; pancreas; ruminant; small intestine

1. Introduction

Maternal nutrition during gestation is a major determinant of fetal and neonatal growth and development (Caton and Hess, 2010) and nutrient restriction during gestation can have adverse effects on visceral tissues of the dam (Scheaffer et al., 2004) and offspring (Meyer et al., 2013; Yunusova et al., 2013; Peine et al., 2018). Alterations in fetal visceral organ function during the prenatal phase can have negative effects on postnatal growth and development (Yunusova et al., 2013). The pancreas and small intestine have important roles in post-ruminal nutrient digestion, and digestive enzymes produced in these tissues respond to changes in nutrient intake (Kreikemeier et al., 1990; Wang et al., 1998; Swanson et al., 2002). In beef cows, nutrient restriction during mid-to-late-gestation increased fetal α -amylase activity but decreased trypsin activity (Keomanivong et al., 2017a). Similarly, maternal nutrient restriction of ewes during mid- to late-gestation decreased fetal pancreatic trypsin activity (Trotta et al., 2020a). In weaned goats, pancreatic α -amylase activity decreased when fed a dried ryegrass-starter diet for 40 d that contained 60% of the energy and protein content of the control but was then restored after a 60-d nutritional recovery period (Sun et al., 2017).

There is far less information on the response of small intestinal digestive enzymes to maternal nutrient restriction in ruminants. Trotta et al. (2020a) demonstrated that fetal lactase activity increased when gestating ewes were nutrient restricted during either mid- or late-gestation (30 d) while other carbohydrase activities were not influenced. It is unclear if changes in pancreatic or small intestinal digestive enzyme activities *in utero* can influence postpartum digestive function or animal performance. Nutritional regulation of post-ruminal digestive enzymes in ruminants is complex (Swanson et al., 2003) and increasing post-ruminal supply of protein results in increased pancreatic and small intestinal carbohydrase activities (Trotta et al., 2020b). Furthermore, recent studies have investigated the influence of individual amino acids such as leucine (Yu et al., 2014), isoleucine (Liu et al., 2018), phenylalanine (Yu et al., 2013), and glutamic acid (Trotta et al., 2020b) on pancreatic exocrine function in ruminants. Pancreatic trypsin and carboxypeptidase B hydrolyze dietary protein and release lysine and arginine as free amino acids, which increases intraluminal arginine concentrations (Grimble, 2007). Increasing dietary levels of arginine results in increased hepatopancreatic α -amylase and trypsin activities in the Jian carp (Chen et al., 2012). Arginine administration through jugular infusion had no influence on pancreatic α -amylase activity in nonpregnant ewes (Keomanivong et al., 2017b). However, it is unclear if maternal arginine supplementation could potentially influence offspring digestive enzyme

activity in the pancreas or small intestine of ruminants. Therefore, the objective of this experiment was to determine the effects of maternal nutrient restriction and rumen-protected arginine supplementation on post-ruminal digestive enzyme activity of lamb offspring.

2. Materials and Methods

All animal care and sample collection procedures were approved by the North Dakota State University Animal Care and Use Committee.

2.1. Experimental design

The experimental design and description of animal procedures were previously described by Peine et al. (2018). Briefly, 31 multiparous Rambouillet-cross ewes (Initial BW = 67.7 ± 6.2 kg) were bred to rams of proven fertility and pregnancy was confirmed via ultrasound on day 41 ± 6.0 d after mating. Ewes were housed in a climate-controlled environment with *ad libitum* access to water and fed a pelleted diet (DM basis: 37.2% neutral detergent fiber, 21.5% acid detergent fiber, and 15.5% crude protein) that was formulated to meet or exceed requirements for vitamins and minerals (NRC, 1985). Targeted metabolizable energy (ME) requirements were based on NRC (1985) recommendations for 60-kg pregnant ewes during mid- to late-gestation (140 g/d of ADG) and were adjusted to 2.36 Mcal of ME/d based on previous experiments using similar animals, diets, and housing (Yunusova et al., 2013).

Ewes were randomly assigned to 1 of 3 dietary treatments: 100% of dietary requirements (control, CON; $n = 11$), 60% of control (restricted, RES; $n = 10$), or RES with the addition of a rumen-protected arginine (Kemin Industries, Des Moines, IA) supplement (RES-ARG; $n = 10$). The supplement provided RES-ARG ewes with 180 mg rumen-protected arginine•kg BW⁻¹•d⁻¹ (based on initial BW). The rumen-protected arginine was mixed with 50 g of fine ground corn and fed once daily at 0800 before offering the pelleted diet. Control and RES ewes were also provided 50 g of fine ground corn daily. Treatments began on day 54 ± 3.9 d of gestation and continued until parturition. Pelleted diets were fed once daily and were consumed within 2 h. Body weights were measured every 7 d and dietary allotments were adjusted accordingly.

Immediately after parturition, lambs were removed from dams and reared independently. Lambs were fed artificial colostrum (Lifeline Rescue Colostrum, APC, Ankeny, IA) at 0, 4, 6, 12, 16, and 20 h post-partum to achieve 10.64 g IgG/kg of lamb birth weight, according to the methods described previously (Meyer et al., 2010a; Neville et al., 2010). At 24 h after birth, lambs were fed milk replacer (Super Lamb Milk Replacer, Merrick's Inc., Middleton, WI; DM basis: 30% crude fat and 24% crude protein) via a bottle and were gradually transitioned to a teat bucket system (Meyer et al., 2010a; Neville et al., 2010). Lambs had access to a mixture of long-stem mid-bloom alfalfa hay and creep feed (DM basis: 20% crude protein, 8% crude fiber, and 6% crude fat). Milk-replacer and the hay-creep feed mixture were offered for *ad libitum* intake.

2.2. Sample collection

At day 54 ± 3 d of age, lambs were slaughtered via captive bolt stunning and exsanguination. The pancreas and small intestine were isolated, trimmed of mesentery and

adipose tissue, and weighed. Identifying and separating the jejunum was conducted similar to the methods of Soto-Navarro et al. (2004). A 15-cm transverse section of jejunal tissue was subsampled, cut longitudinally, and rinsed with phosphate-buffered saline. The pancreas and jejunal subsamples were weighed, and then the jejunal mucosa was scraped using a glass microscope slide (Siddons, 1968). The pancreatic and jejunal subsamples were flash-frozen in isopentane (2-Methylbutane, J. T. Baker, Center Valley, PA) that was super-cooled in liquid nitrogen and then stored at -80°C until further analyses (Keomanivong et al., 2016).

2.3. Digestive enzyme activity

Digestive enzyme activity was determined using approaches similar as described previously (Swanson et al. 2002; Trotta et al. 2020b). Briefly, pancreatic (250 ± 4.81 mg) and jejunal (505 ± 21.4 mg) tissue were weighed and diluted with 2.25 mL and 2.0 mL of 9 g/L sodium chloride solution, respectively. Pancreatic and jejunal samples were homogenized (Kinematica Polytron PT 10/35; Brinkmann Instruments Inc.) and protein concentration was measured using the bicinchoninic acid (BCA) procedure (Pierce BCA Protein Assay Kit, Cat no. 23225; Thermo Fisher Scientific Inc.) using bovine serum albumin as the standard (Smith et al., 1985). Activity of α -amylase was determined using the procedure from Wallenfels et al. (1978) using a commercially available reagent (Amylase Reagent Set, Cat no. A533; Teco Diagnostics) containing p-nitrophenyl-D-maltoheptaoside as the substrate. Pancreatic trypsin activity was assayed kinetically using the methods described by Geiger and Fritz (1986) using N-alpha-Benzoyl-DL-arginine-4-nitroanilide hydrochloride (CAS: 911-77-3; Thermo Fisher Scientific Inc.) as the substrate after activation with enterokinase (CAS: 9014-74-8; Sigma-Aldrich Co.; Glazer and Steer, 1977).

The jejunal segment was assayed for lactase, maltase, isomaltase, sucrase, and glucoamylase were assayed using the modified methods of Dahlqvist (1964) with lactose, maltose, isomaltose, and soluble starch used as the substrates, respectively, in a potassium phosphate buffer (Turner and Moran, 1982) and 100 μL of 60 mM substrate solution in a 1.5-mL centrifuge tube. Tubes were incubated for 30 min at 39°C in a water bath. The reaction was terminated by heating tubes for 2 min in a 90°C water bath, followed by drenching in an ice bath. Tubes were then centrifuged at $4000 \times g$ for 20 min at 4°C . Liberated glucose was measured using the hexokinase/glucose-6-phosphate dehydrogenase procedure (Farrance, 1987).

Analyses were adapted for use on a microplate spectrophotometer (Synergy H1; BioTek Instruments, Inc.) at 39°C . One unit (U) of pancreatic digestive enzyme activity equals 1 μmol of p-nitrophenol for α -amylase or 1 μmol of p-nitroaniline for trypsin per min. Pancreatic digestive enzyme activity data are expressed as U/g pancreas (activity per gram pancreas), U/g protein (activity per gram protein), kU/pancreas (total activity), and U/kg BW (activity relative to BW). One unit (U) of jejunal digestive enzyme activity equals 1 μmol of glucose produced per min for lactase, glucoamylase, and sucrase and 0.5 μmol of glucose produced per min for maltase and isomaltase. Jejunal digestive enzyme activity data are expressed as U/g jejunum (activity per gram jejunum) and U/g protein (activity per gram protein).

2.4. Statistical analysis

Data were analyzed for normality and homogeneity of variances. All data were analyzed as a completely randomized design for effects of treatment using the GLM procedure of SAS (SAS 9.4, Cary, NC) with lamb as the experimental unit. Because there were 4 sets of twins (Peine et al., 2018), fetal number was initially included in the model statement but was removed because there were no effects ($P > 0.10$) on any of the variables measured. Fetal sex was also initially included in the model but was removed because there were no effects ($P > 0.10$) on any of the variables measured. Contrast statements were used to determine differences between means for effects of restriction (CON vs. RES and RES-ARG) and rumen-protected arginine supplementation (RES vs. RES-ARG). Results were considered significant if $P \leq 0.05$. Tendencies were declared when $0.05 < P \leq 0.10$.

3. Results

Maternal nutrient restriction and rumen-protected arginine supplementation did not influence pancreatic protein concentration or α -amylase activity per gram pancreas, activity per gram protein, total activity, or activity relative to BW (Table 1). Pancreatic trypsin activity per gram protein tended to be greater ($P = 0.08$) in lambs exposed to maternal nutrient restriction from midgestation to parturition. Maternal nutrient restriction did not influence trypsin activity per gram pancreas, total activity, or activity relative to BW. Maternal rumen-protected arginine supplementation did not influence pancreatic trypsin activity. The α -amylase:trypsin was not influenced by treatment.

Jejunal protein concentration was not influenced by either maternal nutrient restriction or rumen-protected arginine supplementation (Table 2). No treatment effects were observed for jejunal enzymes involved in starch digestion including maltase, isomaltase, and glucoamylase. Maternal nutrient restriction tended to decrease ($P = 0.09$) lactase activity per gram protein but did not influence lactase activity per gram jejunum. Maternal rumen-protected arginine supplementation did not influence jejunal lactase activity. Sucrase activity was undetected in the jejunum of lamb offspring.

4. Discussion

Post-ruminal digestive enzymes production can respond to changes in nutrient intake through changes in activity per gram of tissue (Kreikemeier et al., 1990) or changes in tissue mass (Wang et al., 1998; Swanson et al., 2002a). Maternal nutrient restriction tended to decrease pancreatic mass of lamb offspring in the current study (Peine et al., 2018). In contrast, Meyer et al. (2013) found that pancreatic mass of neonatal lambs (20 d) from nutrient restricted ewes tended to be greater than lamb pancreatic mass from ewes fed at 140% of NRC requirements from mid-gestation to parturition. Maternal nutrient restriction of ewes from mid-gestation to parturition had no influence on lamb offspring small intestinal mass on day 20 (Meyer et al., 2013) or day 180 (Yunusova et al., 2013) postpartum. Maternal nutrient restriction did not influence small intestinal mass, mucosal density, or crypt cell proliferation of lamb offspring used in the current study at day 54 postpartum (Peine et al., 2018).

Previous research from our laboratory has shown that maternal nutrient restriction influences fetal pancreatic and intestinal digestive enzyme activity in ruminants. Nutrient restriction of ewes during mid- to late-gestation had no effect on fetal pancreatic α -amylase or trypsin activity (Keomanivong et al., 2016). However, maternal nutrient restriction of beef cows during early- or early- to mid-gestation decreased trypsin activity from fetal pancreas collected during late-gestation (Keomanivong et al., 2017a). Similarly, fetal pancreatic trypsin activity was decreased in response to maternal nutrient restriction of ewes during mid- to late-gestation (Trotta et al., 2020a). In the current study, maternal nutrient restriction from mid-gestation to parturition tended to increase pancreatic trypsin activity of lamb offspring. This may indicate that maternal nutrient restriction may result in increased trypsin activity as a compensatory growth mechanism to compensate for inadequate prenatal nutrient supply and deficient production of trypsin during fetal development. Indeed, maternal nutrient restriction decreased lamb BW at birth but BW did not differ among treatments at day 54 (Peine et al., 2018). In support of this hypothesis, Neville et al. (2010) reported that lambs from ewes restricted from mid-gestation to parturition did not differ in post-weaning total tract N digestibility or N balance, suggesting that decreases in fetal trypsin activity may not affect the capacity for adequate protein digestion during postnatal life (Trotta et al., 2020a). Because lambs had *ad libitum* access to milk-replacer, creep feed, and hay in the current study, it is possible that lambs from RES or RES-ARG treatments consumed more than CON lambs. However, if feed intake did increase, then it would be expected that other digestive enzyme activities would increase as well.

Pancreatic (α -amylase) and small intestinal (maltase, isomaltase, and glucoamylase) carbohydrases contribute to luminal and membrane-bound hydrolysis of starch to di- and oligosaccharides to glucose. Shifting the site of starch digestion from the rumen to the small intestine can increase energetic efficiency up to 42% (Owens et al., 1986). However, small intestinal starch digestion in ruminants is potentially limited by deficient production of carbohydrases (Owens et al., 1986; Brake and Swanson, 2018). Thus, research on nutritional strategies to increase digestive enzyme activities are warranted. Maternal nutrient restriction of ewes during mid- to late-gestation did not influence fetal pancreatic α -amylase, or small intestinal maltase, isomaltase, and glucoamylase activities (Trotta et al., 2020a). In the current study, maternal nutrient restriction of ewes did not influence enzymes involved in small intestinal starch digestion in lamb offspring at 54 d of age. Similarly, jejunal maltase activity at day 180 postpartum did not differ in offspring from nutrient restricted ewes (Yunusova et al., 2013). Collectively, these data suggest that maternal nutrient restriction should not be used as a developmental programming strategy to increase fetal brush border carbohydrase activities in sheep.

In the current study, nutrient restriction of gestating ewes tended to decrease fetal lactase activity in the jejunum. A forty-percent maternal nutrient restriction during mid-gestation (day 50 to 90 of gestation) and realimentation to 100% of NRC requirements during late gestation (day 90 to 130 of gestation) increased fetal lactase activity in sheep (Trotta et al., 2020a). In the same study, fetal lactase activity did not differ among animals that were exposed to either 60% or 100% of NRC requirements during mid-to-late-gestation (day 50 to 130). Thus, reasons for the lesser activity of lactase in the fetal small intestine in RES and RES-ARG treatments are unclear because ewes in both studies had similar diets, housing,

and extent and length of nutrient restriction. Even if milk-replacer, creep feed, or hay intake differed between treatments, these factors are thought to be independent of influencing lactase activity in weaning lambs (Shirazi-Beechey et al., 1991). Likewise, development of the reticulorumen is not associated with changes in small intestinal digestive enzyme expression or activity (Shirazi-Beechey et al., 1991). Indeed, stomach complex mass (reticulorumen, omasum, and abomasum) was decreased in RES and RES-ARG lambs in the current study (Peine et al., 2018). Smith and James (1987) speculated that increases in crypt cell proliferation could potentially inhibit lactase expression by 1) shortening the time for lactase expression and 2) decreasing the rate of lactase synthesis. However, this may not apply to the current study because crypt cell proliferation did not differ among treatments (Peine et al., 2018). Immunological status of the gut may influence the capacity for lactase activity in humans (Phillips et al., 1988), and this could potentially be a contributing factor to changes in small intestinal biology with maternal nutrient restriction. Additional data such as intestinal morphology, mRNA expression of enzymes, or protein abundance across multiple sites of the intestine could be useful for elucidating factors influencing digestive enzyme activity.

Maternal rumen-protected arginine supplementation did not influence lamb offspring pancreatic or jejunal digestive enzyme activities. Post-ruminal supply of amino acids such as glutamic acid (Trotta et al., 2020b) and leucine (Reiners et al., 2019) have been shown to influence small intestinal maltase activity in cattle. In a previous study, jugular administration of 155 μmol arginine/kg of BW to nonpregnant ewes had no influence on pancreatic mass, activity of α -amylase or trypsin, or serum insulin concentration (Keomanivong et al., 2016). Although rumen-protected arginine supplementation increases duodenal flow of arginine and apparent small intestinal disappearance of arginine (g/d) in steers (Meyer et al., 2018), metabolism by maternal visceral tissues and/or the placenta could potentially reduce the amount of supplemental arginine available for fetal uptake.

5. Conclusions

Maternal nutrient restriction tended to increase pancreatic trypsin activity of lamb offspring. Maternal nutrient restrictions from mid-gestation to parturition tended to decrease jejunal lactase activity of lamb offspring. Maternal rumen-protected arginine supplementation had no influence on pancreatic or jejunal digestive enzyme activities of lamb offspring. These data suggest there is little influence of maternal nutrient restriction or rumen-protected arginine supplementation on post-ruminal digestive enzyme activity of lamb offspring with *ad libitum* intake postpartum.

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Highlights

- Nutrient restriction of ewes did not influence starch digesting enzymes of lambs.
- Feeding rumen-protected arginine did not influence carbohydrases of lambs.
- Minimal effects on maternal programming of digestive enzymes were observed.

Table 1.

Influence of maternal nutrient restriction and rumen-protected arginine supplementation on pancreatic digestive enzyme activity in lambs.

	Treatment			SEM ^a	P-value	
	CON	RES	RES-ARG		Restriction ^b	Arginine ^c
Protein						
mg/g pancreas	116	118	118	4.2	0.78	0.95
g/pancreas	3.43	2.95	3.12	0.242	0.15	0.65
mg/kg BW	143	134	132	10.3	0.36	0.90
α -Amylase						
U/g pancreas	12.8	15.3	15.8	2.09	0.25	0.85
U/g protein	105	130	132	16.0	0.17	0.90
U/pancreas	375	399	424	72.2	0.66	0.80
U/kg BW	15.5	17.7	18.1	3.12	0.50	0.93
Trypsin						
U/g pancreas	2.94	3.80	3.63	0.438	0.13	0.78
U/g protein	24.7	32.2	30.5	3.23	0.08	0.71
U/pancreas	86.3	94.1	99.9	16.62	0.56	0.79
U/kg BW	3.60	4.25	4.31	0.752	0.41	0.94
α -Amylase:trypsin	4.12	4.26	4.55	0.521	0.65	0.71

Abbreviations: CON = control; RES = restricted; RES-ARG = restricted + rumen-protected arginine supplementation.

^aStandard error of the mean (CON, n = 11; RES, n = 10; RES-ARG, n = 10).

^bRestriction = CON vs. RES and RES-ARG.

^cArginine = RES vs. RES-ARG.

Table 2.

Influence of maternal nutrient restriction and rumen-protected arginine supplementation on jejunal digestive enzyme activity in lambs.

	Treatment			SEM ^a	P-value	
	CON	RES	RES-ARG		Restriction ^b	Arginine ^c
Protein, mg/g	82.5	89.7	123.3	20.1	0.35	0.25
Glucoamylase						
U/g jejunum	1.23	1.54	2.13	0.335	0.12	0.22
U/g protein	14.6	21.3	21.4	4.32	0.18	0.99
Isomaltase						
U/g jejunum	2.04	2.20	2.53	0.199	0.16	0.24
U/g protein	26.0	30.3	27.1	4.29	0.58	0.60
Lactase						
U/g jejunum	23.4	21.8	20.0	1.94	0.33	0.52
U/g protein	302	269	215	27.9	0.09	0.19
Maltase						
U/g jejunum	2.57	2.90	3.38	0.456	0.28	0.46
U/g protein	32.3	43.1	35.1	9.20	0.51	0.53

Abbreviations: CON = control; RES = restricted; RES-ARG = restricted + rumen-protected arginine supplementation.

^aStandard error of the mean (CON, n = 11; RES, n = 10; RES-ARG, n = 10).

^bRestriction = CON vs. RES and RES-ARG.

^cArginine = RES vs. RES-ARG.