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The influence of pregnancy and plane of nutrition during pregnancy on pancreatic digestive enzymes and insulin-containing cell cluster morphology in beef cows

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Running Head: Pregnancy, intake, and pancreatic function in cows

To determine effects of pregnancy (Experiment 1) and plane of nutrition during pregnancy (Experiment 2) on pancreatic digestive enzymes and morphology of insulin-containing cell clusters, beef cows were slaughtered and the pancreas collected 4 wk before parturition (if pregnant or similar time on feed for non-pregnant cows). In Experiment 1, mature, non-lactating cows (717 ± 70 kg; 9 pregnant [PREG] and 9 non-pregnant [OPEN]) were used. In Experiment 2, non-lactating, pregnant cows (639 ± 68 kg) were fed at 85% ($n = 11$; LOW) or 140% ($n = 11$; HIGH) of NE requirements. Concentration (U/g) and content (U/kg BW) of α -amylase and trypsin activity were greater ($P \leq 0.05$) in OPEN than PREG cows. Pregnant cows had greater ($P \leq 0.03$) insulin-cell cluster size and proportion of large insulin-containing cell clusters than OPEN cows. Concentration and content of α -amylase activity was greater ($P \leq 0.04$) in HIGH than LOW cows. These data indicate that pregnancy status (PREG vs. OPEN) impacts pancreatic exocrine and endocrine function and that plane of nutrition (85 vs 140% of NE requirements) of pregnant cows may not greatly impact pancreatic exocrine and endocrine function.

Keywords: Cow, Dietary intake, Digestive enzymes, Insulin, Pancreas, Pregnancy

Abbreviations: BW, body weight; HIGH, 140% of NE requirements; LOW; 85% of NE requirement; NE, net energy; OPEN; non-pregnant: PREG, pregnant, U, unit.

The pancreas has both exocrine and endocrine functions and is important in regulating digestion and metabolism (Chandra and Liddle 2011). Pancreatic enzymes such as lipase, trypsin, and α -amylase are secreted into the small intestine where they are important for digestion of lipids, protein, and starch, respectively (Sissons 1981) and hormones, such as insulin, also are secreted systemically that help to regulate whole-animal metabolism (Begg and Woods 2012).

Because of the structure of the stomach in ruminants, large amounts of pre-gastric fermentation occurs (Russell and Hespell 1981). This ruminal fermentation impacts the flow of nutrients to the small intestine. As a result of pre-gastric fermentation, ruminants often have limited net portal appearance of glucose relative to short-chain fatty acids (Bell and Bauman 1997; De Koster and Opsomer 2013) and adult cattle are often fed lower quality diets that are high in fiber to capitalize on this fermentative digestive system. Because of this altered supply of nutrients to the small intestine, the role of pancreatic digestive enzymes likely differs between ruminant and non-ruminant species. Ruminants also rely largely on gluconeogenesis from substrates such as propionate and alanine to supply glucose to the body (Bell and Bauman 1997). Therefore, the pancreas in ruminants potentially may adapt to changes in physiological state or diet differently than non-ruminants. Pregnancy results in metabolic adaptations of the dam for proper fetal development and to maintain body condition (Bell and Bauman 1997). Pregnancy results in increased demand for glucose and other nutrients and the pancreas is a key tissue in modulating these demands (Lain and Catalano 2007).

The mechanisms by which pancreatic digestive enzymes are regulated by diet and physiological state in ruminants are not well understood (Swanson and Harmon 2002).

Dietary energy intake is thought to be an important regulator of pancreatic exocrine function in growing cattle (Kreikemeier et al. 1990; Swanson et al. 2008b). However, less is known in mature cows about the regulation of pancreatic exocrine function.

The endocrine role of the ruminant pancreas is thought to be similar to non-ruminants, although because of ruminal fermentation, the substrates influencing endocrine function likely differ (Mayhew et al. 1969). For example, besides glucose, short chain fatty acids and protein also are thought to have a stimulatory effect on insulin secretion in ruminants (Harmon, 1992). Less is known about the effects of physiological status or nutrition on pancreatic endocrine morphology in ruminants, which likely is important in the production of hormones in the pancreas.

We hypothesize that both pregnancy status and dietary energy intake during gestation will influence pancreatic exocrine enzymes and morphology of insulin-containing clusters in mature cows. Thus, the objective of this experiment was to determine the effect of pregnancy (Experiment 1) and feed intake (Experiment 2) on pancreatic α -amylase, trypsin, and lipase activities and content and morphology of insulin-containing clusters in mature beef cows.

MATERIALS AND METHODS

Animal Management and Treatments

The experiments were approved by the University of Guelph Animal Care Committee and the animals were cared for according to the guidelines of the Canadian Council of Animal Care (1993). Animal care and experimental design was described previously (Wood et al. 2013a; Wood et al. 2013b).

For Experiment 1 (Wood et al. 2013b), 18 mature ($n = 9$ non-pregnant [OPEN] and $n = 9$ pregnant [PREG]) cows primarily of Angus and Simmental crossbreeding were used in a replicated, randomized complete block design to determine the effects of pregnancy on pancreatic function. Cows were 5.33 ± 2.8 yr old and went through at least one successful pregnancy before start of the trial. Cows were blocked according to expected day of gestation, such that PREG cows in each block were slaughtered at approximately 4 to 5 wk before parturition and OPEN cows were randomly assigned to block. At the start of the trial, PREG cows were approximately at 150 to 165 d of gestation. Cows were fed once daily a total mixed ration containing grass haylage (69.5% of diet DM), wheat straw (30% of diet DM), and a trace mineral and vitamin supplement (0.5% of diet DM; 35.8% NaCl, 14% Na, 12% Ca, 4% P, 1% Mg, 0.6% S, 0.2% K, 2369 mg/kg Mn, 1000 mg/kg Cu, 3000 mg/kg Zn, 2294 mg/kg Fe, 58 mg/kg I, 25.5 mg/kg Co, 16.2 mg/kg Se, 601.5 KIU/kg vitamin A, 100.5 KIU/kg vitamin D, and 2000 IU/kg vitamin E). Cows were fed for ad libitum intake and individual feed intakes were measured using Calan gates (American Calan, Inc., Northwood, NH), with orts measured at least once per wk. Cows were weighed every 28 d. Final BW was obtained the morning of slaughter. To accommodate differing stages of gestation of PREG, experimental design was arranged as a replicated randomized complete block design so that tissues were collected at approximately 250 d of gestation. The first replicate contained three blocks of four cows (two cows from each treatment), where the first block was slaughtered on d 89 of the feeding period, and blocks 2 and 3 slaughtered 7 and 14 d, respectively, after block 1. The second replicate contained two blocks (block 1 and 3) and contained six cows, four cows (two per treatment) in block 1 and 2 cows (one

in each treatment) in block 3 and were slaughtered on the same schedule as the first replicate.

For Experiment 2 (Wood et al. 2013a), twenty-two (11 per dietary treatment) non-lactating, mature pregnant beef cows, primarily of Angus and Simmental crossbreeding were used in a randomized complete block design. Each block ($n = 5$ or 6) was slaughtered approximately 4 wk before expected date of parturition (approximately 250 d of gestation). Cows were multiparous, averaged 3.23 ± 1.04 (mean $\pm$ SD) yr old and had previously gone through at least one successful pregnancy. Cows were randomly assigned to pen ($n = 12$) and 1 of 2 dietary treatments: high level of feed intake ($n = 11$; HIGH): formulated to be $1.4 \times$ total NE requirements for maintenance and fetal growth (NRC 1996), equivalent to 2.1% of BW; and low level of intake ($n = 11$; LOW): formulated to be $0.85 \times$ total NE requirements for maintenance and fetal growth, equivalent to 1.25% of BW. Dry matter intake was measured for individual animals using Calan gates (American Calan, Inc., Northwood, NH), with orts (if present) collected once per week. Feed intakes were adjusted for individual cows every 14 d based on BW to maintain a constant level of NE intake relative to treatment (1.4 or $0.85 \times$ total NE requirements for maintenance and fetal growth). Diets were fed once daily as a total mixed ration (TMR) and contained grass haylage (79.5% of diet DM), wheat straw (20% of diet DM), and a trace mineral and vitamin supplement (0.5% of diet DM; 35.8% NaCl, 14% Na, 12% Ca, 4% P, 1% Mg, 0.6% S, 0.2% K, 2,369 mg/kg Mn, 1,000 mg/kg Cu, 3,000 mg/kg Zn, 2,294 mg/kg Fe, 58 mg/kg I, 25.5 mg/kg Co, 16.2 mg/kg Se, 601.5 KIU/kg vitamin A, 100.5 KIU/kg vitamin D, and 2,000 IU/kg vitamin E). Four cows from blocks 1 through 4 and 6 (2 from each treatment) and 2 cows from block 5 (1 from

each treatment) were slaughtered at the University of Guelph Meat Laboratory on each sample collection day. The first block of cows was slaughtered on d 83 relative to the start of dietary treatments, and the remaining blocks were slaughtered weekly thereafter, such that cows were sent to slaughter at a common day of gestation (approximately d 250 of gestation). Cows were not fasted before slaughter and were fed their experimental diet the d before slaughter.

Tissue Collection

Pancreata were removed, trimmed of mesentery and fat, and weighed (Swanson et al. 2002a; Swanson et al. 2008a). A subsample from the body of the pancreas was collected, frozen in liquid nitrogen, and stored at -80°C until analysis for α -amylase, trypsin, and lipase activities and protein concentration. A subsample of pancreatic tissue was also fixed in 10% formalin and later embedded in paraffin blocks to be used in immunohistochemistry analyses (Keomanivong et al. 2015).

Pancreatic Enzyme Analysis

Pancreatic tissue (1g) was homogenized in 0.9% NaCl (9 mL) using a polytron (Brinkmann Instruments Inc, Westbury, NY). Activity of α -amylase was determined by the procedure of Wallenfels et al. (1978) using a commercially available kit (Teco Diagnostics, Anaheim, CA). Trypsin activity was assayed using the method described by Geiger and Fritz (1986) after activation (Glazer and Steer 1977; Swanson et al. 2002a) with 100 U/L enterokinase (Sigma-Aldrich, Oakville, ON., Canada). Pancreatic lipase activity was measured using a colorimetric method (Imamura and Misaki 2014) and a commercially available kit (Point Scientific, Inc., Canton, MI). Protein concentration (mg/g) was determined (Smith et al. 1985) using a bicinchoninic acid protein assay kit

(Pierce, Rockford, IL) with bovine serum albumin used as the standard. Analyses for digestive enzyme activity and protein concentration were adapted for use on a PowerWave XS microplate spectrophotometer (BioTek Instruments Inc., Winooski, VT). Enzyme activity data are expressed as units (U) per gram of wet tissue (U/g), units per gram of protein (U/g protein), kilounits per pancreas (kU/pancreas), and units per kilogram of BW (U/kg BW). One U of enzyme activity equals 1 mole product produced per min.

Immunohistochemistry and Histological Analysis

Pancreatic tissue collected from a representative area of the body of the pancreas was immersion fixed in 10% formalin and embedded in paraffin blocks with proper and persistent orientation as described previously (Borowicz et al. 2007; Keomanivong et al. 2015). From each sample, 5- μ m tissue sections were obtained from the blocks and mounted on slides. Tissue sections on the slides were then deparaffinized and rehydrated. Slides were incubated in the staining enhancer citrate buffer heated at 95°C for 5 min then cooled to room temperature. Slides were then covered in a solution of 10% donkey serum and 1% bovine serum albumin diluted in tris-buffered saline for a period of 20 min. Slides were incubated with 1:100 mouse anti-pig insulin (Santa Cruz Biotechnology, Dallas, TX) in 1% BSA and tris-buffered saline overnight at 4°C on a plate rocker. The following day, the slides were washed with tris-buffered saline with added tween 20 (TBST) and incubated with 1:100 CFTM633 Goat Anti-Mouse IgG secondary antibody (Biotium, Inc, Hayward, CA) for one hour in complete darkness. A final wash with TBST was performed and coverslips were applied using mounting

medium containing 4',6- diamidino-2-phenylindole in order to visualize all pancreatic cell nuclei.

Photomicrographs were taken with an Imager.M2 epifluorescence microscope (Carl Zeiss Microscopy, Jena, Germany) using a 10x objective and AxioCam HRc camera with a piezo automated stage. To describe morphology of the pancreas, the mosaic image of a large tissue area of approximately 144 pictures (12×12 pictures) on the slide was taken using the MosaiX module of AxioVision software (Carl Zeiss Microscopy). This method allowed creation of a single image of tissue area of approximately 72 mm^2 . The MosaiX images were then analyzed using the ImagePro Premier software (ImagePro Premier 9.0, Media Cybernetics, Silver Spring, MD) for the insulin positive area per section of tissue. The images were analyzed by creating regions of interest corresponding to the whole area of pancreatic tissue visible on the picture. Within the regions of interest, images were segmented based on positive insulin staining and measurements of total positive insulin staining within pancreatic tissue (%) and number of insulin-containing cell clusters were determined (Figure 1). Insulin-containing cell clusters were further classified based on size in order to perform population density measurements of small clusters ($1 - 2,000 \mu\text{m}^2$), medium clusters ($2,001 - 32,000 \mu\text{m}^2$) and large clusters ($32,001 - 512,000 \mu\text{m}^2$). Insulin-positive cell clusters did not correspond to islets of Langerhans as not every cell in the islet is insulin positive.

Statistical Analysis

Data were analyzed using PROC MIXED (SAS Inst. Inc., Cary NC) of SAS. For Experiment 1, the model included the effect of treatment, cow age and block nested

within replicate. For Experiment 2, the model included the effect of dietary treatment, cow age, pen and block. Pen nested within block and treatment was included as a random effect. Results were considered significant at $P \leq 0.05$ and a tendency when $0.05 < P < 0.10$.

RESULTS

Experiment 1

Final body weight (BW) did not differ between OPEN and PREG cows (Table 1) as reported previously (Wood et al. 2013b). Pancreas weight (g and g/kg), and protein concentration and content (mg/g and g/pancreas), did not differ between OPEN and PREG cows. Pancreatic protein content relative to BW (g/kg BW) tended ($P = 0.06$) to be greater in OPEN than PREG cows. Concentration (U/g and U/g protein) and content (kU/pancreas and U/kg BW) of α -amylase were greater ($P \leq 0.005$) in OPEN than PREG cows (Table 2). Concentration (U/g and U/g protein) and content (U/pancreas and U/kg BW) of trypsin activity were also greater ($P \leq 0.05$) in OPEN than PREG cows. Concentration (U/g and U/g protein) of lipase activity tended ($P = 0.06$) to be greater in PREG than OPEN cows but content (kU/pancreas and U/kg BW) of lipase activity did not differ between treatment. Pancreatic insulin-positive area (% of tissue section analyzed) did not differ between OPEN and PREG COWS (Table 3; Figure 1). However, PREG cows had a greater ($P = 0.03$) average insulin-containing cell cluster size as well as a greater ($P = 0.02$) proportion of large insulin-containing cell clusters and a greater ($P = 0.02$) largest insulin-containing cell cluster per cow.

Experiment 2

Final BW was greater ($P = 0.04$) for HIGH than LOW cows (Table 4) as reported previously (Wood et al. 2013a). Pancreas weight (g and g/kg BW) did not differ between treatments. Protein concentration (mg/g) and content relative to BW (mg/kg BW) were not influenced by treatment but total content (g/pancreas) was greater ($P = 0.05$) in HIGH than LOW cows. Concentration (U/g and U/g protein) and content (kU/pancreas, U/kg BW) of α -amylase activity was greater ($P \leq 0.04$) in HIGH than LOW cows (Table 5). There were no differences in trypsin and lipase activities between LOW and HIGH cows. No differences were observed for insulin positive area, islet size distribution, total number of islets and average islet size between treatments (Table 6; Figure 1).

DISCUSSION

Experiment 1

Little is known about the effect of physiological state (including pregnancy) on pancreatic exocrine and endocrine function in ruminants. Our results indicate that OPEN cows had greater α -amylase and trypsin activity compared to PREG cows. Research has suggested that pancreatic protein secretion increases in pregnant compared with non-pregnant dogs (Rosenberg et al. 1975). Also, serum α -amylase and lipase did not differ in pregnant vs. non-pregnant women and with advancing gestation (Ordorica et al. 1991), although the physiological significance of endocrine secretion of digestive enzymes is not well characterized and serum concentrations of digestive enzymes do not necessarily reflect pancreatic content or secretion into the digestive tract (Isenman et al. 1999).

The physiological significance of altered digestive enzyme concentration and content in the pancreas of mature cows is unclear. Pancreatic α -amylase secretion has been suggested to potentially limit post-ruminal starch digestion in growing cattle fed

high-grain diets when there is significant starch flow to the small intestine (Swanson and Harmon 2002). Post-ruminal starch flow to the small intestine for the cows in the current experiment was likely very low as the diet did not contain feeds containing significant starch concentrations, such as grains, making the need for greater production of α -amylase likely unnecessary. Therefore, the biological significance of differences in pancreatic α -amylase concentration or content in cows from the current experiment is not clear. Less is known regarding limitations in protein digestion in the small intestine resulting from inadequate production and secretion of proteolytic enzymes, such as trypsin. Lipid flow to the small intestine also was likely very low in this experiment as the diet did not contain feeds containing significant lipid concentrations. The increase in pancreatic α -amylase and trypsin concentration and content in OPEN cows as compared to PREG could have occurred because these cows were in more of a positive energy balance because of the lack of need for energy and nutrients for fetal development. Carcass ultrasound fatness measurements (rib fat and rump fat) were not different between treatments (Wood et al., 2013b). However, carcass marbling score tended to be greater in OPEN vs. PREG cows (Wood et al., 2013b) suggesting that cows were at different energy status. Differences in energy balance between OPEN and PREG cows is also supported by the increase in NEFA, urea N, and β -hydroxybutyrate in PREG vs. OPEN cows and no differences in DMI and ADG between treatments (Wood et al., 2013b). Past research in growing cattle has suggested that dietary energy is an important regulator of pancreatic exocrine function (Kreikemeier et al. 1990; Swanson et al. 2008b).

During pregnancy, the insulin demands of maternal tissues commonly increase (Lain and Catalano 2007). In humans, beta cell mass and cell size increase in response to pregnancy (Rieck and Kaestner 2010) which agrees with our results on pancreatic insulin-containing clusters in this experiment. This alteration in pancreatic morphology is thought to occur because of increased metabolic demand for insulin because of increased demands and decreased insulin sensitivity (Lain and Catalano 2007). This response has also been reported to occur in pregnant beef cows (Sano et al. 1991). These effects are believed to be the result of the actions of pregnancy hormones (Nieuwenhuizen et al. 1997). Progesterone receptors are expressed on endocrine but not exocrine cells of the human pancreas (Doglioni et al. 1990). Some data suggests, however, that the effects of progesterone on islet cell proliferation is an indirect effect and may be due to gonadal factors other than estradiol (Nieuwenhuizen et al. 1999). In any case, it seems that the endocrine pancreas in cows responds to pregnancy similarly to non-ruminants such as rats (Nieuwenhuizen et al. 1997) and humans (Lain and Catalano 2007).

Experiment 2

In contrast to monogastric species, pancreatic secretion in ruminants seems to be more continuous with little increase or decrease relative to a feeding event (Taylor 1962; McCormick and Stewart 1967; Croom, Jr. et al. 1992). In growing ruminants, nutrition influences pancreatic digestive enzyme content and secretion (Swanson and Harmon 2002). Past research in growing cattle has suggested that dietary energy is an important regulator of pancreatic exocrine function (Kreikemeier et al. 1990; Swanson et al. 2008b). However, ruminants may respond to substrate differently than monogastric

species. For example, pancreatic α -amylase content and secretion decreases with increasing starch flow to the small intestine (Swanson et al. 2002a; Swanson et al. 2002b) whereas in monogastric species dietary starch typically increases pancreatic α -amylase content and secretion (Brannon 1990). In the current experiment increasing dietary intake, and thus net energy intake, resulted in increased α -amylase but no difference in trypsin and lipase concentration and content. The reasons for the differential regulation of α -amylase vs. trypsin and lipase are unclear.

Changes in physiological state as well as nutrition may likewise have a significant impact on the pancreatic concentration of insulin. Previous work has indicated that diets low in protein result in reduced pancreatic insulin concentration (Dahri et al. 1995). Other data has suggested increasing plane of nutrition results in greater concentration of systemic plasma concentration of insulin (Schoenberg and Overton 2011). The reasons we did not observe an effect of dietary intake on the content and morphology of insulin-containing cell containing clusters is not clear. Perhaps the difference in feed intake between treatments was not great enough to elicit an effect or other factors than changes in insulin-cell cluster content or morphology were mediating changes in metabolism resulting from differing plane of nutrition.

CONCLUSION

Activity of pancreatic α -amylase and trypsin decreased and the size of pancreatic insulin-cell clusters increased in PREG as compared to OPEN cows. Plane of nutrition generally did not influence digestive enzyme activity, except for increased concentration and content of α -amylase activity in LOW vs. HIGH cows, and pancreatic insulin-cell cluster amount and morphology. These data indicate that pregnancy status (PREG vs.

OPEN) impacts pancreatic exocrine and endocrine function and that plane of nutrition of pregnant cows may not greatly impact pancreatic exocrine and endocrine function.

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Table 1. Final BW, DMI, ADG, plasm metabolites, and pancreatic weight and protein in open and pregnant cows.

Item	Treatment ^a		SEM	<i>P</i>
	OPEN	PREG		
Final BW ^b , kg	784	845	26.9	0.12
DMI ^b , kg/d	12.8	13.4	0.36	0.25
ADG ^b , kg/d	0.75	0.90	0.08	0.19
Final plasma NEFA ^b , mmol/L	0.23	0.47	0.077	0.04
Final plasma urea ^b , mmol/L	3.47	4.00	0.16	0.03
Pancreas				
g	612	561	40.3	0.36
g/kg	0.778	0.661	0.0496	0.10
Pancreatic protein				
mg/g	101	101	3.3	0.89
g/pancreas	61.5	55.4	3.58	0.23
g/kg BW	0.078	0.065	0.0046	0.06

^aOPEN = non-pregnant cows (*n* = 9); PREG = pregnant cows (*n* = 9).

^bFrom Wood et al. (2013b)

Table 2. Digestive enzymes in open and pregnant cows.

Item	Treatment ^a		SEM	<i>P</i>
	OPEN	PREG		
α -Amylase				
U/g	200	113	18.6	0.005
U/g protein	1963	1071	156.0	0.001
kU/pancreas	120	59	10.1	0.001
U/kg BW	154	70	12.9	<0.001
Trypsin				
U/g	5.21	3.33	0.629	0.05
U/g protein	51.8	31.5	5.79	0.02
U/pancreas	3135	1673	348.7	0.01
U/kg BW	4.05	2.01	0.40	0.003
Lipase				
U/g	17.5	21.1	1.28	0.06
U/g protein	173	216	14.96	0.06
kU/pancreas	10.9	11.7	1.22	0.60
U/kg BW	13.86	13.94	1.59	0.97

^aOPEN = non-pregnant cows ($n = 9$); PREG = pregnant cows ($n = 9$).

Table 3. Pancreatic insulin positive staining area and islet size distribution in open and pregnant cows.

Item	Treatment ^a		SEM	<i>P</i>
	OPEN	PREG		
Pancreatic insulin- positive tissue area (relative to section of tissue), %	0.40	0.60	0.150	0.34
Insulin-containing cell clusters within the following ranges, %				
Small clusters (1 – 2,000 µm ²)	75.7	67.7	2.91	0.07
Medium clusters (2,001 – 32,000 µm ²)	24.2	30.4	2.92	0.16
Large clusters (32,001 – 512,000 µm ²)	0.04	1.90	0.475	0.02
Average size of insulin-containing cell clusters, µm ²	2315	3731	403	0.03
Smallest insulin-containing cell clusters, µm ²	259	253	2.94	0.14
Largest insulin-containing cell clusters, µm ²	27,416	59,177	8,583	0.02

^aOPEN = non-pregnant cows (*n* = 9); PREG = pregnant cows (*n* = 9).

Table 4. Final BW, DMI, ADG, plasma metabolites, and pancreatic weight and protein of cows fed above or below total net energy requirements.

Variable	Treatment ^a		SEM	<i>P</i>
	HIGH	LOW		
Final BW ^b , kg	738	690	12.7	0.04
DMI ^b , kg/d	10.9	6.8	0.119	<0.001
ADG ^b , kg/d	1.11	0.59	0.066	0.003
Final plasma NEFA ^b , mmol/L	0.47	1.08	0.144	0.03
Final plasma urea ^b , mmol/L	3.30	3.87	0.175	0.07
Pancreas				
g	468	380	36.3	0.14
g/kg BW	0.638	0.561	0.0545	0.34
Pancreatic protein				
mg/g	96.1	95.6	5.51	0.95
g/pancreas	44.7	35.0	2.87	0.05
mg/kg BW	60.5	52.6	3.65	0.15

^aHIGH = cows fed at $1.4 \times$ total NE requirements ($n = 11$); LOW = cows fed at $0.85 \times$ total NE requirements ($n = 11$).

^bFrom Wood et al. (2013a).

Table 5. Pancreatic digestive enzyme activity of cows fed above or below total net energy requirements.

Variable	Treatment ^a		SEM	<i>P</i>
	HIGH	LOW		
α -Amylase				
U/g	113.2	66.2	12.48	0.04
U/g protein	1125	712	91.5	0.02
kU/pancreas	53.4	22.2	6.09	0.01
U/kg BW	69.5	35.1	9.65	0.04
Trypsin				
U/g	2.56	2.23	0.358	0.54
U/g protein	26.4	23.2	2.50	0.42
U/pancreas	1190	833	190.0	0.22
U/kg BW	1.60	1.22	0.276	0.35
Lipase				
U/g	13.4	11.6	1.19	0.34
U/g protein	145	122	15.6	0.34
kU/pancreas	6.14	5.13	0.553	0.23
U/kg BW	8.33	7.45	0.744	0.47

^aHIGH = cows fed at 1.4 × total NE requirements (n = 11); LOW = cows fed at 0.85 × total NE requirements (n = 11).

Table 6. Pancreatic insulin positive staining area and islet size distribution of cows fed above or below total net energy requirements.

Variable	Treatment ^a		SEM	<i>P</i>
	HIGH	LOW		
Pancreatic insulin-positive tissue area (relative to section of tissue), %	0.813	0.709	0.2778	0.80
Insulin-containing cell clusters within the following ranges, %				
Small clusters (1 – 2,000 μm^2)	66.9	64.4	5.09	0.74
Medium clusters (2,001 – 32,000 μm^2)	33.4	33.6	4.26	0.98
Large clusters (32,001 – 512,000 μm^2)	0.63	1.77	1.122	0.51
Average size of insulin-containing cell clusters, μm^2	2748	3952	1137	0.48
Smallest insulin-containing cell clusters, μm^2	257	252	4.7	0.48
Largest insulin-containing cell clusters, μm^2	51,851	41,921	17,009	0.70

^aHIGH = cows fed at $1.4 \times$ total NE requirements ($n = 11$); LOW = cows fed at $0.85 \times$ total NE requirements ($n = 11$).

Figure 1. Effects of pregnancy and level of feed intake on insulin-positive staining. Sections represent the mature pregnant (a) and open (b) beef cattle pancreatic tissues along with cattle receiving high (c) vs low (d) feed intake pancreatic tissues. White areas represent insulin-containing cells. Magnification 10x.

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Figure 1. Effects of pregnancy and level of feed intake on insulin-positive staining. Sections represent the mature pregnant (a) and open (b) beef cattle pancreatic tissues along with cattle receiving high (c) vs low (d) feed intake pancreatic tissues. White areas represent insulin-containing cells. Magnification 10x.

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