The impact of diet and arginine supplementation on pancreatic mass, digestive enzyme activity and insulin-containing cell cluster morphology during the estrous cycle in sheep

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Abbreviations used: UF (Underfed), CON (control), OF (overfed), Arg (treated with arginine),
Sal (saline), BCS (body condition score), TBST (buffered-saline with added tween 20)

Abstract

To determine the effect of feed intake and arginine treatment during different stages of the estrous cycle on pancreatic mass, digestive enzyme activity, and histological measurements, ewes ($n = 120$) were randomly allocated to one of three dietary groups; control (CON; 2.14 Mcal metabolizable energy/kg), underfed (UF; 0.6 x CON) or overfed (OF; 2 x CON) over 2 yr. Estrus was synchronized using a controlled internal drug release (CIDR) device for 14 d. At CIDR withdrawal, ewes from each dietary group were assigned to one of two treatments; Arg (L-Arg HCl, 155 $\mu\text{mol/kg BW}$) or Sal (approximately 10 mL Saline). Treatments were administered 3 times daily via jugular catheter and continued until slaughter on d 5 and 10 of the second estrus cycle (early luteal phase, $n = 41$ and mid-luteal phase, $n = 39$; year 1) and d 15 of the first estrus cycle (late luteal phase, $n = 40$; year 2). A blood sample collected from jugular catheters for serum insulin analysis before slaughter. The pancreas was then removed, trimmed of mesentery and fat, weighed, and a sample snap-frozen until enzyme analysis. Additional pancreatic samples were fixed in 10% formalin solution for histological examination of size and distribution of insulin-containing cell clusters. Data were analyzed as a completely randomized design with a factorial arrangement of treatments. Diet, treatment, and diet $\times$ treatment were blocked by year and included in the model with initial BW used as a covariate. Day of the estrous cycle was initially included in the model but later removed as no effects ($P > 0.10$) were observed for any pancreatic variables tested. Overfed ewes had the greatest ($P < 0.001$) change in BW, final BW, change in BCS, and final BCS. A diet $\times$ treatment interaction was observed for change in BW and final BW ($P \leq 0.004$). Overfed and CON had increased ($P < 0.001$) pancreas weight (g)

compared to UF ewes. Protein concentration (g/pancreas) was lowest ($P < 0.001$) in UF ewes while protein content (mg/kg BW) was greater ($P = 0.03$) in UF than OF ewes. Activity of α -amylase (U/g, kU/pancreas, U/kg of BW, and U/g protein) and trypsin (U/pancreas) was greater ($P \leq 0.003$) in OF than UF ewes. Serum insulin was greatest ($P < 0.001$) in OF ewes. No effects were observed for pancreatic insulin-containing cell clusters. This study demonstrated that plane of nutrition affected several measurements of pancreatic function however, the dosage of Arg used did not influence pancreatic function.

Keywords: sheep, arginine, feed intake, pancreas, enzymes

1. Introduction

One of the biggest challenges in animal production is providing adequate feed with proper nutrient profiles during each stage of life. Sheep breeders are often faced with this dilemma as grazing flocks can at certain times of the year receive less than 50% of NRC recommendations [1]. Periods of nutrient restriction impact production and secretion of pancreatic digestive enzymes [2]. A decrease in the secretion of pancreatic digestive enzyme activity due to reduced feed intake influences the breakdown and absorption of nutrients in the small intestine [3,4].

Arginine is known to play a role in tissue function and enzyme activity [5]. These functions are impacted by the ability of arginine to stimulate secretion of hormones such as insulin, growth hormone, glucagon and prolactin, and to act as a precursor of proteins, polyamines and nitric oxide (NO) which becomes a powerful vasodilator and biological regulator of tissues [1,5,6]. Based on these understandings, arginine supplementation has been indicated as

a possible rescue factor for detrimental effects caused by nutrient restriction on organ function [7].

During different stages of life, the animal endures reduced or excess intake and understanding the effect of diet on the pancreas is crucial. The concentration of pancreatic insulin varies throughout the estrus cycle and also depends on plane of nutrition [8,9,10]. In addition, it has been demonstrated that pancreatic α -amylase has been influenced by nutritional factors capable of modification by steroid sex hormones [11,12]. Therefore, sex hormones exert further effects beyond the targeted measurements of reproductive function [10,13,14]. The interaction between day of estrus and variable feed intake or supplemental arginine on pancreatic function is unclear.

We hypothesized that Arg-treatment will affect selected pancreatic functions in non-pregnant ewes fed inadequate diets compared to ewes fed a maintenance control diet. Therefore, the objectives were to determine the effects of different levels of feed intake and arginine supplementation on pancreatic mass and protein concentration, activity of α -amylase and trypsin, insulin-containing cell cluster measurements, and serum insulin concentration at the early-, mid-, and late-luteal phases of the estrous cycle.

2. Materials and methods

This experiment was approved by the North Dakota State University (NDSU) Institutional Animal Care and Use Committee.

2.1 Animal dietary groups

Animal use and experimental design have been previously described [10]. Non-pregnant, non-lactating, individually-fed Rambouillet ewes ($n = 120$) between 2 and 5 yr of age and of similar genetic background were studied over 2 yr. Ewes weighed 60.2 kg ($\pm$ a standard deviation of 5.79 kg) with an initial body condition score of 2.7 ($\pm$ a standard deviation of 0.24) on a 1 to 5 scale. Ewes were housed in a temperature-controlled environment at 14°C and a 12:12 light:dark cycle with lights on at 07:00 and off at 19:00 each day. Sixty days prior to the beginning of the experiment ewes were stratified by BW and randomly allocated to one of three dietary groups; control (CON; 100% National Research Council [15] requirements; 2.4 Mcal metabolizable energy/kg BW), underfed (UF; 0.6 x CON) or overfed (OF; 2 x C; Table 1). These respective diets were provided twice daily at 08:00 and 15:00 for the duration of the study and were adjusted weekly based on ewe BW to ensure that proper body condition was achieved. The jugular vein of each ewe was cannulated prior to the initiation of the estrous cycle [10]. Estrus was synchronized using a controlled internal drug release (CIDR) device for 14 d.

2.2.1 Saline and Arg treatments

Approximately 36 hr after CIDR withdrawal, ewes were considered in estrus and were randomly allocated to one of two treatments; Arg (L-Arg HCl, 155 μ mol/kg BW) or Sal (~10 mL Saline) [10]. Ewes receiving Sal were given 5 to 10 mL of sterile saline solution via the jugular catheter, whereas ewes receiving Arg were given 5 to 10 mL of L-Arg-HCl solution in a dose of 155 μ mol/kg BW (Sigma St. Louis, MO, USA). Sal or Arg treatments were initiated on d 0 of the first estrous cycle and occurred three times daily (07:00, 14:00, and 21:00) until the end of the experiment. The dose of Arg was selected based on studies performed by Lassala et al [7,16]

to ensure an increased concentration of Arg in maternal serum. After treatment injection, 1 mL of heparin solution was placed into the catheter to prevent clotting.

2.3 Blood and tissue collection

At the early- (d 5; $n = 41$) or mid- (d 10; $n = 39$) luteal phase of the second estrous cycle (Year 1) and at the late-luteal phase (d 15; $n = 40$) of the first estrus cycle (Year 2), final BW and BCS were recorded and blood was collected from the jugular catheter using 9-mL Luer Monovette blood collection tubes (Sarstedt, Newton, NC, USA). After collection, blood samples were allowed to clot for 15 to 20 min at room temperature and centrifuged (Allegra 6R Centrifuge; Beckman Coulter Inc., Indianapolis, IN, USA) for 20 min at $3500 \times g$. Serum was then aliquoted into 2-mL serum tubes and immediately stored at -20°C until further analysis.

Ewes were stunned via captive bolt and exsanguinated. The pancreas was removed, trimmed of mesentery and fat, and a total weight recorded. A sample from the body of the pancreas was snap-frozen in isopentane super-cooled in liquid nitrogen until analyses for protein concentration, and α -amylase and trypsin activities. Additional samples of pancreatic tissue were fixed in 10% formalin solution and later embedded in paraffin blocks to be used in immunohistochemistry analyses including quantitative characterization of size and distribution of insulin-containing cell clusters.

2.3.1 Serum insulin concentration

Insulin concentrations in 100 μL of serum were measured with a chemiluminescent immunoassay on an Immulite 1000 analyzer (Siemens Healthcare, Tarrytown, NY, USA), as previously described and validated (including specificity) by Vonnahme et al (17). Briefly, the

Immulite 1000 test units contain assay-specific antibody-coated polystyrene beads. Once the protein of interest is bound to the antibodies on the bead, it is detected by the binding of an alkaline phosphatase-labeled, assay-specific antibody. Dioxetane is the chemiluminescent alkaline phosphatase substrate, and the amount of emitted light is proportional to the amount of analyte in the sample. Biorad Lymphochek standards were used to validate the assay with levels 1, 2 and 3 control pools containing low, intermediate and high levels of insulin respectively (Biorad, Hercules, CA, USA). Samples were assayed in duplicate using Immulite 1000 Insulin kits (catalog # LKIN1), and the intra-assay CV was 13.2%. The reportable range for the assay is 2-300 uIU/mL, and the analytical sensitivity is 2 uIU/mL.

2.4 Pancreatic protein, α -amylase and trypsin activity analyses

Pancreatic tissue (0.25 g) was homogenized in 0.9% NaCl (2.25 μ l) using a polytron (Brinkmann Instruments Inc., Westbury, NY, USA). Protein concentration was determined using the bicinchoninic acid (BCA) procedure with bovine serum albumin (BSA) used as the standard [18]. Activity of α -amylase was determined using the procedure of Wallenfels [19] utilizing a kit from Teco Diagnostics (Anaheim, CA, USA). Trypsin activity was determined using the methods described by Geiger and Fritz [20] after activation with 100 U/L enterokinase [21]. Analyses were adapted for use on a microplate spectrophotometer (SpectraMax 340, Molecular Devices, Sunnyvale, CA, USA). One unit (U) of enzyme activity equals 1 μ mole product produced per min. Enzyme activity data are expressed as U/g wet tissue, U/g protein, kU/pancreas, and U/kg BW.

2.5 Immunohistochemistry

A representative sample of the body of the pancreas was immersion-fixed in formalin and embedded in paraffin blocks with proper and persistent orientation, and immunohistochemistry was performed as described previously [3,22]. Tissues were cut (5- μ m sections), mounted on slides, deparaffinized and rehydrated. Slides were incubated in staining enhancer citrate buffer heated to 95°C for 5 min then cooled to room temperature. Slides were covered in a solution of 10% donkey serum and 1% bovine serum albumin diluted in tris-buffered saline for 20 min then incubated with 1:100 mouse anti-pig insulin (Santa Cruz Biotech, Dallas, TX, USA) primary antibody in 1% BSA and tris-buffered saline at 4°C on a plate rocker for 24 hr. Next, 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 (Santa Cruz Biotech, Dallas, TX, USA) for one hr 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. Negative control slides were prepared with omitting primary antibody.

2.6 Histological analysis

Photomicrographs were taken with a Zeiss Imager.M2 epifluorescence microscope (Carl Zeiss Microscopy, Jena, Germany) using a 10x objective and AxioCam HR camera with a Zeiss piezo automated stage. To describe morphology of the pancreas, the mosaic image of a large tissue area of approximately 144 pictures (12 $\times$ 12 pictures) on the slide was taken using the MosaiX module of Zeiss AxioVision software. This method allowed creation of a single image of the pancreatic tissue present on the slide [3]. The MosaiX images were then analyzed using the ImagePro Premier software (ImagePro Premier 9.0, Media Cybernetics, Silver Spring, MD,

USA) to determine the insulin-positive tissue area per section of tissue, and size of insulin-containing cell clusters. The images were analyzed by creating regions of interest corresponding to the whole area of pancreatic tissue visible on the picture [22]. Within the regions of interest, images were segmented based on positive insulin staining and measurements of total positive insulin staining within pancreatic tissue expressed as percent as well as of individual insulin-containing cell clusters. Insulin-containing cell clusters were further classified based on size to perform population density measurements of small clusters (1 to 2,000 μm^2), medium clusters (2,001 to 32,000 μm^2), and large clusters (32,001 to 512,000 μm^2). Insulin-positive cell clusters do not correspond to islets of Langerhans because not every cell in the islet is insulin positive.

2.7 Statistical analysis

Data were analyzed using the general linear model procedures of SAS 9.2 (Statistical Analysis Software, SAS Institute Inc., Cary, NC, USA) as a completely randomized design with a factorial arrangement including diet, treatment, and diet $\times$ treatment. Data were initially analyzed within year with stage of the estrous cycle initially included in the model for year 1. Stage of the estrous cycle did not influence ($P > 0.10$) any of the variables tested and therefore was removed from the model and the data were combined over all stages and analyzed including year in the model as a blocking factor and initial BW as a covariate. Means were separated using the least significant difference approach and were considered significant when $P \leq 0.05$.

3. Results

Average daily gain and final BW (kg) were greatest ($P < 0.001$) in OF ewes followed by CON (Table 2) while a diet $\times$ treatment interaction was noted with OF ewes treated with Arg having the greatest values for both measurements ($P \leq 0.004$; Figure 1). The changes in BCS and final BCS were greatest ($P < 0.001$) in OF followed by CON ewes while no differences were noted with Arg treatment. Pancreas weight (g) was greater ($P < 0.001$) in OF and CON than UF ewes. Sheep treated with Arg tended to have a greater ($P = 0.09$) pancreas weight (g) than those treated with Sal. Pancreas weight (% of BW) was greater ($P < 0.01$) in UF than OF while CON ewes were intermediate. Arg treated ewes tended to have a greater ($P = 0.09$) pancreas weight (% of BW) than ewes treated with Sal.

No differences ($P > 0.05$) in pancreatic protein concentration (mg/g) were noted between dietary groups or Arg vs Sal treatments. Protein content (g/pancreas) was greater ($P = 0.001$) in CON and OF ewes than UF while protein (% of BW) was greater ($P = 0.03$) in UF than OF with CON intermediate.

The concentration (U/g) of α -amylase activity was greater ($P < 0.001$) in OF and CON than UF ewes. Overfed ewes had the greatest ($P < 0.001$) content of α -amylase activity (kU/pancreas) followed by CON. Both OF and CON had a greater ($P = 0.003$) content of α -amylase activity relative to BW (U/kg BW) than UF ewes. When expressed as U/g protein, the α -amylase activity was greatest ($P < 0.001$) in OF followed by CON and then UF. Arginine treatment had no impact on α -amylase activity.

Trypsin activity (U/g) was not different among treatment groups and when expressed as U/pancreas was greater ($P = 0.003$) in OF than UF with CON not being different from either dietary group. Trypsin activity (U/kg BW and U/g protein) was not different ($P > 0.05$) between diet or Arg treatment.

Serum insulin concentration was greatest ($P < 0.001$) in OF followed by CON and then UF ewes (Table 3). No effects of diet or Arg treatment ($P > 0.05$) were noted in the pancreatic insulin-positive area (relative to section of tissue) or size of insulin-containing clusters (μm^2).

4. Discussion

Changes in BW and BCS due to variable levels of feed consumption are well documented for sheep and other species [10,15,23,24]. Increased change in BW, final BW, and BCS in ewes provided dietary intake similar to that in the current study have been previously reported [3,24] as overfed ewes tended to gain weight while those restricted in nutrient intake lost weight. The diet $\times$ treatment interactions observed for final BW indicated that OF ewes infused with Arg had the greatest final BW compared to other treatments. This may be because of the ability of Arg to increase NO production which is the major vasodilatory factor [10]. This could then allow for increased transport of nutrients ingested by the overfed ewes. However, although serum Arg concentration increased with Arg treatment, serum nitric oxide metabolite concentration did not differ between dietary treatments or with Arg treatment [10] suggesting that these responses may not have been mediated by changes in NO synthesis. Surprisingly, a study involving dairy heifers infused with Arg in the peritoneum demonstrated that the proportion of essential amino acids in plasma was greater in heifers exposed to a control rather than an Arg- treatment [25]. Infusing the ewes directly into the jugular vein in the current study however, may play a role in the differences observed, as may the difference in the amount of arginine used.

The UF ewes in this study had lower pancreatic weight (g). This is likely the result of reduced metabolizable energy and nutrient intake thus resulting in decreased tissue growth

[3,21,26]. Furthermore, because BW was subjected to a greater decrease than pancreatic weight it is not surprising that UF ewes had a greater pancreatic weight (% of BW) than OF ewes [27].

Increasing dietary intake has been associated with greater pancreatic protein concentration (g/pancreas) and the secretion of exocrine digestive enzymes in this and other studies [28]. It has been reported that enhanced pancreatic protein concentration may increase the hydrolysis of nutrients in the small intestine [2,4,29,30].

An increase in the content (U/pancreas) of α -amylase and trypsin activity in OF ewes was observed in the current study which is likely due to increased feed intake [28,31] resulting in increases in enzyme concentration (U/g pancreas) or increased tissue mass (g) of the pancreas and therefore tissue enzyme content. Because no difference in the concentration of trypsin (U/g) was noted in this experiment, the increase in content of trypsin activity was likely due to increased tissue mass whereas the increase in α -amylase content was due to increases in both enzyme concentration and tissue mass.

Insulin concentration in serum was greater in OF than CON and UF ewes in the current study. Similarly, greater insulin concentration in overfed and lower insulin concentration in underfed animals were reported by others [10,23,32-35]. In this study serum insulin concentration was not affected by treatment with Arg. In addition, Arg-treatment during late pregnancy did not affect plasma insulin concentration in ovine fetuses [36]. In contrast, high doses of Arg increased insulin concentration in ovine fetuses at late pregnancy, and Arg supplementation increased insulin concentration in calves [37,38]. Furthermore, Arg infusion caused an acute increase in insulin concentration in ovine fetuses and dairy cows [39]. These data indicate that Arg effects on insulin production depends on dose, reproductive status and

species. On the other hand, direct associations between feed intake and serum insulin concentration is well documented for several species [3].

The morphology of insulin containing cell cluster sizes was not affected by diet or Arg-treatment. Although NO has been previously shown to cause changes in exocrine and endocrine function of the monogastric pancreas, it's function on ovine pancreatic function seems to be less clear [6,10,40]. It is also possible that pancreatic morphology of the ewes was unaltered due to the fact that the animals were mature and pancreatic development had already been achieved. While differences have been observed in our previous studies involving pregnant ewes [3], the amount of time spent under nutrient restriction was significantly longer and there may be different responses within pregnant and non-pregnant ewes.

In the present and our previous study [10], pancreatic measurements and/or insulin concentration were not affected by stages of the estrous cycle that is characterized by changes of progesterone secretion. These results may be due to the fact that the days examined are close enough that pancreatic enzyme activity and insulin-cluster morphology were not different.

In summary, results obtained from the current experiment have demonstrated that diet but not Arg-treatment or stage of the estrous cycle affected pancreatic function including mass, digestive enzyme activity, and serum insulin concentration. These results are supported by the findings in the companion study of Kaminski et al. [10] who noted a greater impact of diet than Arg or stage of the estrous cycle on measurements of serum metabolites (glucose) and selected hormones (insulin-like growth factor 1, leptin, and progesterone). Thus, contrary to our hypothesis, dose and duration of Arg-treatment used was ineffective as a therapeutic to overcome compromised nutrition in ewes, but plane of nutrition seems to be a major regulator of pancreatic exocrine function.

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436

Table 1. Dietary Composition and Nutrient Analysis

Ingredient (% of Diet DM)	Diet DM %
Beet pulp	36.5
Alfalfa meal	22.3
Corn	18.2
Soy hulls	20.0
Soybean meal	3.0
<u>Nutrient Composition</u>	
DM, %	91.78
OM, % of DM	93.71
CP, % of DM	11.57
NDF, % of DM	37.36
ADF, % of DM	25.27
Calcium, % of DM	0.87
Phosphorus, % of DM	0.27

Table 2. Influence of nutrient restriction and arginine treatment on BW, BCS, pancreatic mass and digestive enzymes in non-pregnant ewes

Item	Dietary Group			SEM	Treatment		SEM	<i>P</i> -Values		
	UF	CON	OF		Saline	Arg		Diet	Treatment	Diet × Treatment
^a Change in BW (kg)	-13.6	-1.13	7.92	0.811	-2.44	-2.09	0.686	< 0.0001	0.71	0.003
Final BW (kg)	46.6 ^c	59.1 ^b	68.1 ^a	0.811	57.8	58.1	0.69	<0.0001	0.71	0.003
^a Change in BCS (Score 1-5)	-0.488 ^c	0.482 ^b	1.21 ^a	0.0713	0.410	0.390	0.0603	<0.0001	0.81	0.67
Final BCS	2.24 ^c	3.17 ^b	3.91 ^a	0.0800	3.10	3.12	0.068	<0.0001	0.84	0.39
Pancreas Wt										
g	58.0 ^b	68.5 ^a	72.7 ^a	2.12	64.3	68.5	1.79	<0.0001	0.09	0.28
% of BW	1.25 ^a	1.17 ^{ab}	1.07 ^b	0.039	1.12	1.20	0.033	0.004	0.09	0.88
Protein										
mg/g	120	124	120	2.7	123	120	2.3	0.46	0.33	0.49
g/pancreas	7.02 ^b	8.46 ^a	8.74 ^a	0.339	7.92	8.23	0.293	0.0006	0.44	0.19
mg/kg BW	151 ^a	144 ^{ab}	128 ^b	6.5	138	144	5.6	0.03	0.47	0.85
α -Amylase										
U/g	91.5 ^b	136 ^a	168 ^a	11.73	136	128	9.9	<0.0001	0.57	0.86
kU/pancreas	5.15 ^c	9.21 ^b	12.7 ^a	0.946	9.14	8.88	0.799	<0.0001	0.82	0.92
U/kg of BW	113 ^b	156 ^a	184 ^a	15.1	153	150	12.7	0.003	0.87	0.96
U/g protein	749 ^c	1099 ^b	1359 ^a	87.0	1090	1049	74.3	<0.0001	0.69	0.75
Trypsin										
U/g	9.74	9.95	10.9	0.657	10.2	10.2	0.55	0.41	0.94	0.54
U/pancreas	567 ^b	672 ^{ab}	791 ^a	47.7	648	702	40.2	0.003	0.31	0.89
U/kg BW	12.1	11.5	11.8	0.85	11.3	12.3	0.72	0.89	0.30	0.72
U/g protein	80.9	93.4	90.7	5.80	84.7	85.3	4.89	0.43	0.94	0.35

*Data are presented as least square means per treatment $\pm$ SEM. Different letters signify means are different ($P < 0.05$). Underfed, UF (n = 42); Control, CON (n = 39); Overfed, OF (n = 39); Arginine, Arg (n = 59); Saline (n = 61). ^aOver the experimental period (5 point scale)

Table 3. Influence of nutrient restriction and arginine treatment on serum insulin and measurements of insulin containing clusters in pancreas in non-pregnant sheep.

Item	Dietary Group			SEM	Treatment		SEM	<i>P</i> -Value		
	UF	CON	OF		Saline	Arg		Diet	Treatment	Diet × Treatment
Serum Insulin	6.97 ^b	9.88 ^b	13.2 ^a	1.142	10.8	9.19	0.977	0.001	0.24	0.88
Pancreatic insulin-positive area (relative to section of tissue), %	1.42	1.30	1.31	0.1486	1.39	1.29	0.124	0.82	0.58	0.66
Insulin-containing clusters within the following ranges (µm ²), %										
1 – 2,000	64.5	67.7	64.7	1.97	64.8	66.5	1.64	0.43	0.48	0.67
2,001 – 32,000	34.9	31.5	34.5	1.88	34.4	32.8	1.55	0.37	0.47	0.63
32,001 – 512,000	0.607	0.762	0.760	0.2071	0.734	0.685	0.1748	0.83	0.84	0.60
Average size of insulin-containing clusters (µm ²)	2988	3021	3158	253.0	3111	3000	210.3	0.86	0.69	0.73
Smallest insulin-containing cluster (µm ²)	251	251	250	0.5	250	250	0.4	0.37	0.93	0.24
Largest insulin-containing cluster (µm ²)	48936	55686	46492	6790.8	52364	48379	5643.6	0.61	0.62	0.16

*Data are presented as least square means per treatment ± SEM. Different letters signify means are different ($P < 0.05$). Underfed, UF (n = 42); Control, CON (n = 39); Overfed, OF (n = 39); Arginine, Arg (n = 59); Saline (n = 61).

Figure 1. Diet $\times$ Treatment interaction on change in BW and final BW in non-pregnant ewes.

*Data are presented as least square means per treatment $\pm$ SEM. Different letters signify means are different ($P < 0.05$). Underfed (n = 42); Control (n = 39); Overfed (n = 39); Arginine, Arg (n = 59); Saline (n = 61).

Figure 2. Influence of diet and treatment on insulin-positive staining. Sections represent pancreatic tissues of ewes receiving each diet and treatment. Magnification 10x. UF-Sal (Underfed Saline), UF-Arg (Underfed Arginine), CON-Sal (Control Saline), CON-Arg (Control Arginine), OF-Sal (Overfed Saline), OF-Arg (Overfed Arginine).