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Accepted Version

Relling, A. E., Loerch, S. C. and Reynolds, C. K. ORCID: https://orcid.org/0000-0002-4152-1190 (2014) Intravenous glucagon like peptide-1 infusion does not affect dry matter intake or hypothalamic mRNA expression of neuropeptide Y, agouti related peptide and proopiomelanocortin in wethers. Canadian Journal of Animal Science, 94 (2). pp. 357-362. ISSN 1918-1825 doi: 10.4141/cjas2013-121 Available at https://centaur.reading.ac.uk/87104/

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To link to this article DOI: http://dx.doi.org/10.4141/cjas2013-121

Publisher: Agricultural Institute of Canada

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Intravenous Glucagon Like Peptide-1 Infusion Does Not Affect Dry Matter Intake or Hypothalamic mRNA Expression of Neuropeptide Y, Agouti Related Peptide and Proopiomelanocortin in Wethers.

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ABSTRACT

The objectives of the present study were to determine effects of jugular vein infusions of glucagon like peptide-1 (GLP-1) and dietary fat inclusion on dry matter intake, nutrient digestibility and hypothalamic mRNA concentration of neuropeptide Y, agouti related peptide, and proopiomelanocortin in growing sheep. Thirty six wethers were used (40.7 ± 3.3 kg BW). Treatments were a control diet (n = 11), dietary addition (6% of dry matter) of Ca salts of palm oil fatty acids (n = 12), or 6-d jugular vein infusions of 0.155 μg/kg body weight/day of GLP-1 (n = 11). Hormone concentrations were measured in jugular vein plasma from samples taken on day 1, 4 and 6. On d 7, the wethers were slaughtered for hypothalamus collection to measure

mRNA concentration. The dietary addition of 6% of Ca salts of palm oil increased plasma GLP-1 concentration ($P < 0.01$) and decreased dry matter intake on day 1, but not on day 6 (time x treatment interaction, $P < 0.05$). The infusion of GLP-1 did not change dry matter intake ($P > 0.20$), but increased neutral detergent fibre digestibility ($P < 0.01$). In conclusion, glucagon like peptide-1 infusion or feeding fat did not decrease dry matter intake or affect hypothalamic neuropeptide mRNA concentrations of sheep.

RÉSUMÉ

Les objectifs de cette étude étaient d'évaluer l'effet des infusions dans la veine jugulaire du glucagon-like peptide-1 (GLP-1) et de l'addition alimentaire de matières grasses sur l'ingestion de matière sèche (IMS), la digestibilité des nutriments et la concentration de l'ARNm dans l'hypothalamus du neuropeptide Y (NPY), de la protéine agoutie (AgRP), et de la pro-opiomélanocortine (POMC). Trente-six béliers ont été utilisés (40.7 ± 3.3 kg). Les traitements ont été un régime témoin (n 11), addition alimentaire (6% de la MS) de sels de Ca d'acides palmitiques (n12), ou 6 jours (j) d'infusion dans la veine jugulaire de 0.155 µg/kg PC/j de GLP-1 (n 11). Les concentrations d'hormones ont été mesurées dans le plasma de la veine jugulaire des échantillons prélevés le jour 1, 4 et 6. Le jour 7, les béliers ont été abattus pour la collecte de l'hypothalamus pour mesurer la concentration de l'ARNm de NPY, AgRP et POMC. L'ajout de 6% de sels de Ca d'acides palmitiques a augmenté la concentration plasmatique de GLP-1 (P <0,01) et diminué l'lMS du j 1, mais pas du j 6 (interaction de temps x traitement, P <0,05). L'infusion de GLP-1 n'a pas changé l'IMS (P> 0,20), mais a augmenté la digestibilité des fibres au détergent neutre (P <0,01). Il n'y avait aucune différence dans la concentration de l'ARNm de NPY, AgRP ou POMC en raison de l'infusion de GLP-1 ou de l'addition alimentaire de matières

grasses. En conclusion, la seule perfusion intraveineuse de GLP-1 n'a pas diminué l'IMS chez les ovins en croissance.

Mots-clés: glucagon-like peptide-1, ingestion de matière sèche, mouton, neuropeptides hypothalamiques

Running head: Relling et al**.** Glucagon like peptide 1 infusion in sheep

Keywords*:* glucagon-like peptide-1, dry matter intake, sheep, hypothalamic neuropeptide mRNA

Abreviations: AgRP, agouti-related peptide; **CP**, crude protein; **DM**, dry matter; **DMI**, dry matter intake**; FA,** fatty acids; **GLP-1,** glucagon-like peptide-1(7, 36) amide; **ICV,** intracerebroventricular; **NDF**, neutral detergent fibre; **NPY**, neuropeptide Y; **OM**, organic matter; **POMC**, proopiomelanocortin.

INTRODUCTION

In nonruminants, increasing plasma glucagon-like peptide-1(7, 36) amide (GLP-1) concentration decreases feed intake (Turton et al., 1996). In ruminants, an increase in plasma GLP-1 concentration has been associated with a decrease in dry matter intake (DMI) when fat was added to the diet (Relling and Reynolds, 2007; Bradford et al., 2008, Relling et al., 2010). Also, intrajugular infusion of GLP-1 in wethers decreased DMI to a similar extent as feeding fat (Relling et al., 2011). However, the central mechanism of how GLP-1 regulates feed intake is not certain. In fasted rats, intracerebroventricular (ICV) infusion of GLP-1 did not change mRNA concentration for neuropeptide Y (NPY) (Turton et al., 1996). In contrast, Seo et al. (2008)

reported that ICV infusion of GLP-1 decreased NPY and agouti-related peptide (AgRP) and increased proopiomelanocortin (POMC) mRNA concentration in the hypothalamus of fasted rats. In ruminants, *in vitro* culture of sheep hypothalamus in media containing GLP-1 did not change the relative concentration of NPY, AgRP or POMC mRNA (Relling et al., 2012). However, an increase in NPY and AgRP mRNA was associated with an increase in plasma GLP-1 concentration and a decrease in DMI when fat was fed to growing lambs (Relling et al., 2010). There is a paucity of information on the effect of intravenous infusion of GLP-1 on the mRNA concentration for hypothalamic neuropeptides associated with DMI regulation and its association with DMI. Based on the cited literature, we hypothesized that increases in plasma GLP-1 concentration within physiological concentrations, due to continuous jugular vein infusion of GLP-1 or by feeding fat, would decrease DMI. We also hypothesized that decreases in DMI would be associated with changes in hypothalamic gene expression of NPY, AgRP and POMC. Therefore the objective of our study was to determine the effect of a continuous jugular vein infusion of GLP-1 or feeding fat on plasma GLP-1 concentration, DMI, and mRNA concentration of the neuropeptides NPY, AgRP and POMC in growing wethers.

MATERIALS AND METHODS

Animal care followed guidelines recommended in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1998) and procedures used were approved by the Animal Care Committee of the Ohio Agricultural Research and Development Center.

Three weeks before the start of the experiment, 36 Targhee x Hampshire wethers (40.7 ± 3.3 kg BW) were fed a pelleted control diet (Table 1) formulated to meet nutrient requirements

of growing lambs according to the National Research Council (NRC, 1985). The wethers were grouped by weight and housed in three pens with 12 wethers each. Daily rations were provided at 0800 h, and wethers were fed for ad libitum intake of dry matter (DM, 10% refusal) throughout the study. Treatments were 7 d of: 1) control diet (CONT); 2) supplemental dietary fat (Ca-salts of palm oil) at 6% of ration DM (FAT); 3) control diet with intravenous GLP-1 (GLP-1; 0.155 μg/kg BW/d of GLP-1(7-36) amide H6795, Bachem California Inc, CA) in 1 L of saline (0.9% NaCl). These treatments were selected based on previous results in growing lambs (Relling et al., 2011), where the infusion of 0.155 μg/kg BW/d of GLP-1(7-36) amide produced a similar increase in plasma GLP-1 concentration as adding 6% supplemental fat to the diet. The wethers on the CONT and FAT treatments received a control intravenous infusion of 1 L of sterile saline solution daily. The hormone solutions and the saline solutions were made and infused as described in Relling et al. (2011).

The experiment was conducted as a completely randomized block design. Each of the three groups of wethers was considered as a block. Within each block, the 12 wethers were allocated randomly to one of the three treatments (n = 4/treatment). Beginning two weeks before the experiment, the wethers were housed in individual pens. Wethers fed supplemented fat were adapted to fat supplementation for 2 wk before sampling began, with an amount equal to 2% of ration DM fed on day 1 of the adaptation period, 4% on day 2 and 3, and 6% from day 4 onwards. One week before the experiment started, the lambs were moved into metabolic crates as described previously (Murphy et al., 1994) and adapted to procedures used during the sampling week, including feeding, removal of orts, and changing of fecal collection containers. Forty five hours before the experiment started, jugular vein catheters were established as described previously (Relling et al., 2011). Two animals experienced a drop in DMI to less than 50% of the previous day's intake when lambs were moved into the metabolic crates. Therefore, before the infusions started, one wether on the control treatment and one wether on the GLP-1 treatment were removed from the experiment. The continuous infusions were done as described previously (Relling

et al., 2011) and started at 1000 h on day 1 of the experimental period. Briefly, GLP-1 solutions were prepared using 1 L of sterile saline solution (9 g/L of NaCl; VWR International, West Chester, PA). The liter of saline solution was infused at a rate of 0.725 ml/min during 23 h. The wethers fed the control and the fat supplemented diets were intravenously infused with 1 L of sterile saline solution (9 g/L of NaCl). The targeted dose of GLP-1 infused was calculated using a single compartment, first-order kinetic hormone degradation model, based on the equation:

Increase in hormone concentration x 0.693/half life.

The half life used for this equation was 5 min for GLP-1 (Perfetti and Merkel, 2000). The value, 0.693, is the slope of the first order degradation. The target increase for GLP-1 was based on a previous report (Relling et al., 2011).

The bottles with sterile saline solution and those with GLP-1 in solution were kept on ice during the infusion. The infusion line from the bottle to the animal was sterilized using an ethylene oxide (EtO) gas (Cole-Parmer, Vernon Hills, IL). The connection between the bottle with the infusion and the infusion line included a sterile 0.45 μm syringe filter (Whatman International Ltd, Florham Park, NJ).

Between the end of each day's infusion and the start of the following day, the infusion lines were flushed with sterile saline solution (9 g/L of NaCl) and the filters were changed. Feed was offered daily at 1300 h and the refusals were removed and weighed 23 h later at 1200 h. For digestibility and plasma samples, samples were collected and processed as described previously (Relling et al., 2011). Briefly, to measure digestibility, total fecal collection was performed daily during the last 5 d of each experimental period. Five percent of the total daily feces was collected and composited for analysis of DM (100°C oven for 24 h), neutral detergent fibre (NDF, (Ankom^{200} Fiber Analyzer, ANKOM Technology, Fairport, NY), crude protein (CP, Kjeldahl N

x 6.25), fatty acids (FA, Sukhija and Palmquist, 1988), and ash (AOAC 1990) concentration. Blood samples (10 ml) were taken 6 and 8 h after feed was offered on day 1, 4, and 6 of each experimental period. Blood samples were immediately transferred into polypropylene tubes containing solutions of disodium EDTA and benzamidine HCl (1.6 mg and 4.7 mg/ml blood, respectively) and placed on ice. After centrifugation for 25 min at 1800 x *g* and 4°C, plasma was partitioned into individual polypropylene tubes for each analysis to be performed, flash frozen using liquid N_2 within 40 min of sample collection, and stored at -80°C until analyzed. Samples from the infusate were taken after the in line filters during the first sampling time on day 4 to confirm that the infusate contained the correct concentration of GLP-1. Measured GLP-1 concentrations in the infusate were within 98.2% (±3.7, P = 0.798) of targeted concentrations. Concentrations of insulin and GLP-1 were measured using radioimmunoassays as described previously (Reynolds et al., 1989; Benson and Reynolds, 2001). The intra-assay CV averaged less than 12.5% for insulin and less than 11% for GLP-1. Minimum sensitivities (90% of zero standard binding) of the insulin and GLP-1 assays were 0.0027 and 0.001 ng/tube, respectively. Plasma glucose concentration was measured using a colorimetric assay (#1070 Glucose Trinder, Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was measured using microtiter plates and a plate reader in a two-reaction, enzyme based assay (Wako Chemicals USA, Richmond, VA) as described by Johnson and Peters (1993).

The morning of the seventh day of infusions, the lambs were transported 165 km (transport time was 100 min) to an abattoir for hypothalamus collection. It has been previously reported (Relling et al., 2010) that there were no effects of the same transportation routine on the mRNA concentration for the same genes in the hypothalamus of similar lambs (Relling et al., 2010). The hypothalamus was collected within 1 hour after arrival to the slaughter house as

described by Relling et al. (2010). During hypothalamus collection, one sample from a lamb on the GLP-1 treatment was lost due to damage of the brain caused by the captive bolt used at slaughter.

To measure hypothalamic mRNA concentration for NPY, AgRP and POMC, the protocol and primers used were as described by Relling et al. (2010). Briefly, RNA was extracted with TRIzol® (Invitrogen Carlsbad, CA) using procedures recommended by the manufacturer. Concentration of RNA was determined by measuring absorbance at 260 nm. Reverse transcription (RT) PCR was performed as described by Ndiaye et al. (2008). The relative mRNA concentration of NPY, AgRP, and POMC were determined by quantitative RT PCR using the DNA Engine Monitor 2 (BioRad Laboratories, Hercules, CA). Primers for NPY, AgRP and POMC were validated in sheep hypothalamic tissue by cDNA purification and sequencing. Oligonucleotide primers for NPY, AgRP and POMC were obtained from Qiagen Operon Biotechnologies (Alameda, CA). The primer sequences used are described on Table 2. The quantitative RT PCR was run for a maximum of 35 cycles, under the following conditions: denaturing at 94° C for 30 s, annealing at 60° C for 60 s, and extension at 72° C for 60 s. Concentrations of NPY, AgRP and POMC were normalized to cyclophilin B mRNA expression in the same sample to determine the relative mRNA concentrations of NPY, AgRP, and POMC. Homologous standard curves were prepared from purified NPY, AgRP, and POMC cDNA PCR products to calculate the steady-state concentration of NPY, AgRP, and POMC mRNA in triplicate wells for each sample. The PCR amplification products were electrophoretically separated on 1.5% agarose gels and visualized with ethidium bromide. For initial validation, the specific band corresponding to the size of the expected NPY, AgRP, and POMC cDNA fragment

was cut and purified using the QIAquick Gel Extraction Kit (Qiagen Sciences) for sequence confirmation.

The data were statistically analyzed as a complete randomized block design with repeated measurements in time using the MIXED procedure of SAS (Version 9.1, SAS Institute, Cary, NC) and a model testing the random effects of wether and block, and the fixed effect of treatment and time and their interaction. The two daily plasma samples for hormones and metabolites from the three days of sampling in each experimental period were analyzed in the lab individually but the average for each day was used in the statistical analysis. For digestibility and mRNA concentration data, a similar statistical model was used without the effect of time and its interaction. When the time by treatment interaction was significant, the slice option of SAS was used for separation of means. Fisher's protected LSD test was used for means separation at a *P* value of 0.05, for digestibility, mRNA concentration, and when the time by treatment interaction was not significant ($P > 0.10$). Trends were discussed for *P* values between 0.05 and 0.10.

RESULTS

There was a time by treatment interaction for DMI ($P < 0.05$; Figure 1), due to a greater DMI for GLP-1 and control-fed wethers compared with the fat-fed wethers on day 1, but no difference in DMI on day 6 for the three treatments. Metabolizable energy intake and digestibility of DM, CP, FA and organic matter (OM) was not different among the treatments ($P > 0.10$; Table 3). The addition of dietary fat decreased ($P < 0.05$) and there was a trend for GLP-1 infusion to increase ($P < 0.10$) NDF digestibility compared with control fed wethers. Feeding fat or GLP-1 infusion did not change plasma concentrations of insulin and glucose ($P > 0.30$; Table 4) compared with the control wethers. Compared with control wethers, plasma GLP-1

(Figure 2) and NEFA concentrations (Table 4) increased due to additional dietary fat ($P < 0.05$), but were not affected ($P > 0.10$) by GLP-1 infusion. Hypothalamic mRNA concentrations of NPY, AgRP and POMC were not affected by treatments ($P > 0.25$; Table 5).

DISCUSSION

The objective of the experiment was to infuse GLP-1 to achieve a similar plasma concentration as had been previously observed in response to feeding supplemental fat (Relling et al., 2011). We hypothesized that continuous jugular vein infusion of GLP-1 (within physiological concentrations) or feeding fat would decrease DMI. A second objective was to elucidate if the decrease in DMI typically observed when feeding fat was associated with changes in mRNA concentration of the neuropeptides NPY, AgRP and POMC in response to GLP-1 or by other non GLP-1 effects of feeding fat.

In the present study there was an interaction of treatments and days on DMI. Similar amounts of fat or GLP-1 infusion tended to decrease DMI in sheep in previous studies compared with control animals (Reynolds et al., 2006; Relling et al., 2010; Relling et al., 2011). In the present study, wethers fed fat had a smaller DMI on day 1 compared with control wethers. However, fat-fed wethers had an increase in DMI over time, such that by day 6 they had the same DMI as control wethers. Also, wethers infused with GLP-1 started on day 1 with a greater DMI compared with control wethers, and then their DMI tended to decrease toward day 3. As observed in the present study, Relling et al. (2011) reported that dietary inclusion of 6% fat tended to decrease NDF digestibility in sheep. Harvatine and Allen (2006) reported that the inclusion of fat in dairy cow diets decreased ruminal digestibility, but not total tract digestibility of NDF. A possible reason for the decrease in NDF digestibility in wethers fed diets containing increased fat in the present experiment could be because of increased rate of passage, as

observed in sheep fed a similar fat supplemented diet (Relling et al., 2011); however, rate of passage was not measured in the current experiment. The infusion of GLP-1 tended to increase NDF digestibility compared with control-fed wethers. Our assumption was that an increase in NDF digestibility with GLP-1 infusion would be due to a decrease in gut motility and increased retention time of fibre in the rumen and/or hindgut, allowing more time for NDF fermentation by gut microbes. Results of the present study may be because of an increase in digesta retention time, but in a previous study (Relling et al., 2011) GLP-1 infusion at the same rate had no effect on rate of passage or NDF digestibility. In addition, the effect of fat on NDF digestibility observed in the present study was opposite to the effect of GLP-1 infusion, but feeding fat increased plasma concentration of GLP-1. These observations suggest that the effects of fat on NDF digestibility were not due to an increase in GLP-1 concentration for the fat treatment.

Feeding fat increased plasma GLP-1 concentration, but the infusion of GLP-1 did not change plasma GLP-1 concentration compared with control-fed wethers. As shown in Figure 2, infusion of GLP-1 tended to increase plasma GLP-1 concentration on day 4 but then concentrations decreased on day 6. The lack of response of plasma GLP-1 concentration to GLP-1 infusion could be due to a decrease in endogenous secretion into blood, an increased clearance rate, or both; however, we are not aware of studies that can support this assumption. This lack of response of plasma GLP-1 concentration was unexpected and may in part explain the lack of effects of GLP-1 infusion on DMI. However, this lack of response in the GLP-1 infused wethers does not explain the observed increase in NDF digestibility.

As has been observed previously, feeding supplemental fat increased plasma NEFA concentration (Gagliostro and Chilliard, 1991; Relling and Reynolds, 2007), perhaps due to a higher plasma concentration of lipoproteins (Gagliostro and Chilliard, 1991). This increase in

plasma NEFA occurred concurrently with an increase in plasma GLP-1 concentration. However, infusion of GLP-1 did not increase plasma NEFA concentration. The lack of response on plasma NEFA concentration observed in the present study and observed previously (Relling et al., 2011), suggests GLP-1 infusion does not change plasma NEFA concentration.

In the present study, there were no differences in hypothalamic mRNA concentrations for the neuropeptides NPY, AgRP and POMC due to feeding fat or GLP-1 infusion. It has been observed that feeding the same amount of supplemental fat deceases DMI and increases NPY and AgRP (Relling et al., 2011) in growing wethers. The reason for the lack of response of mRNA concentrations for hypothalamic neuropeptides to supplemental fat in the present experiment is not certain. In the case of the GLP-1 infusion treatment, it may have been due to the inability to achieve a sustained increase in plasma concentrations with the dose infused. However, the lack of response on hypothalamic neuropeptide mRNA concentration is also reflected by the lack of differences on DMI observed on day 6. Despite this lack of response of mRNA concentration, the actual neuropeptide concentration or secretion was not measured. It has been observed that changes in the mRNA concentration are associated with changes in the peptide concentration (Kameda et al., 2001); however, we are not aware of any study which has measured the association between mRNA concentration of the neuropeptide and the secretion of its gene product. In conclusion, glucagon like peptide-1 infusion or feeding fat did not decrease dry matter intake or affect hypothalamic neuropeptide mRNA concentrations of sheep.

ACKNOWLEDGMENTS

We are grateful to Dr. H. Zerby, D. O'Diam and the staff of the OSU Meats Laboratory for their assistance with tissue collection, to Drs. J. L. Pate and K. Ndiaye for their help in the

validation of the PCR work, and to P. Tirabasso, G. Lowes and D. Clevenger for technical assistance. Salaries were provided by state and federal funds appropriated to the Ohio Agricultural Research and Development Center, The Ohio State University.

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338 Table 1

339 Formulation and chemical composition of the control diet and fat supplemented diets

	Diet (% of DM)	
Item	Control and GLP-1[z]	Fat
Ingredients		
Alfalfa meal, 17% CP	20.00	20.00
Soy hulls	20.00	20.00
Ground corn	48.59	43.90
Ca salts of palm oil[y]	-	6.00
Soybean meal, 48% CP	8.00	8.09
Urea	0.50	0.60
Limestone	1.50	-
Monosodium phosphate	0.05	0.05
Trace mineral salts	0.50	0.50
Vitamin A (30,000 IU/g)	0.01	0.01
Vitamin D (3,000 IU/g)	0.01	0.01
Vitamin E (44 IU/g)	0.05	0.05
Selenium (200 mg/g)	0.09	0.09
Animal-vegetable fat	0.30	0.30
Ammonium chloride	0.40	0.40

Chemical composition		
NDF	28.01	24.68
CP	14.96	15.75
Ash	5.92	5.16
Total fatty acids	2.88	7.25

[z] Intravenous GLP-1infused

[y] Megalac®, Church and Dwight Co., Inc., Princeton, NJ.

Table 2. Primer sequences used for the reverse transcriptase quantitative PCR

Item	Forward Sequence, 5’ to 3’	Reverse Sequence, 5’ to 3’
NPY[z]	tcagcgctgcgacactacat	gcagagactggagagcaagt
AgRP[z]	cctgaggaagccttattcct	caggattcatgcagccttac
POMC[z]	agtgtcaggacctcaccacg	gctgctgctaccattccga

[z] NPY = Neuropeptide Y; AgRP = Aguti-related peptide; POMC = Proopiomelanocortin.

359 Table 3

360 Dry matter intake (DMI), metabolizable energy intake (MEI) and digestibility of diet

361 components in growing wethers fed a control diet, the control diet plus 6% Ca salts of palm oil

362 (6% Fat), or infused intravenously with 0.155 µg/kg BW/day of GLP-1.

363

	Treatments				
Item	Control	6% Fat	GLP-1	S.E.	*P*
Lambs per treatment	11	12	11		
DMI (kg/d)[z]	1.33	1.29	1.35	0.07	0.83
MEI (Mcal/d)	3.44	3.73	3.58	0.19	0.68
Digestibility (%)					
Dry matter	69.51	69.26	71.51	0.96	0.21
Organic matter	53.34	50.79	49.74	1.45	0.22
Neutral detergent fibre	48.38	40.31[*]	52.39[†]	1.64	0.01
Crude protein	65.44	67.76	67.30	1.00	0.24
Fatty acids	82.09	85.70	80.02	2.00	0.17

364

365 [*] Differs from control, $P < 0.05$.

366 [†] Differs from control, $P < 0.10$.

367 [Z] Time by treatment interaction ($P < 0.05$).

368

Table 4

Plasma hormone and metabolite concentration in growing wethers fed a control diet, the control diet plus 6% Ca salts of palm oil (6% Fat), or infused intravenously with 0.155 µg/kg BW/day of GLP-1. Due to lack of time by treatment interaction values represent average of day 1, 4 and 6.

	Treatments				*P*		
Item	Control	6% Fat	GLP-1	S.E.	Trt[z]	Time	TxT[z]
Lambs per treatment	11	12	11				
Insulin (p*M*)	312	270	270	23	0.34	0.84	0.82
GLP-1[z] (p*M*)	23	34[*]	25	2	0.01	0.44	0.50
Glucose (m*M*)	3.63	3.55	3.57	0.09	0.73	0.22	0.20
NEFA[z] (m*M*)	49.54	77.54[*]	58.58	8.81	0.08	0.97	0.46

[z] Trt= treatment effect; TxT = time by treatment interaction effect; GLP-1= glucagon-like peptide-1 (7-36) amide; NEFA= non esterified fatty acid.

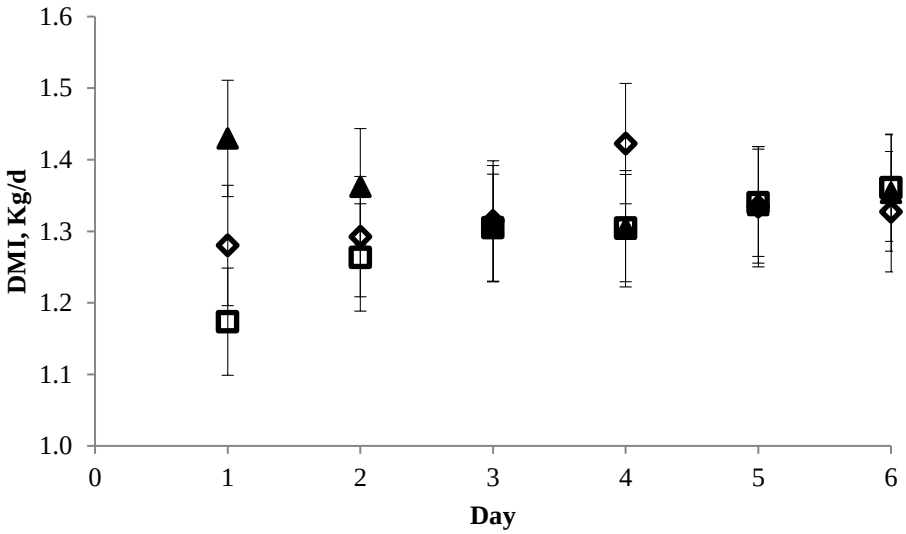
[*] Differs from control, $P < 0.05$.

Table 5

Hypothalamic concentrations of mRNA in growing wethers fed a control diet, the control diet plus 6% Ca salts of palm oil (6% Fat), or infused intravenously with 0.155 µg/kg BW/day of GLP-1.

	Treatments				
Item[z]	Control	6% Fat	GLP-1	S.E.	*P*
Lambs per treatment	11	12	10		
NPY	0.786	0.216	0.137	0.33	0.37
AgRP	0.200	0.031	0.046	0.09	0.40
POMC	0.311	0.168	0.084	0.09	0.25

[z] Concentrations of mRNA (relative to cyclophilin B) for neuropeptide Y (NPY), agouti related peptide (AgRP), and proopiomelanocortin (POMC).

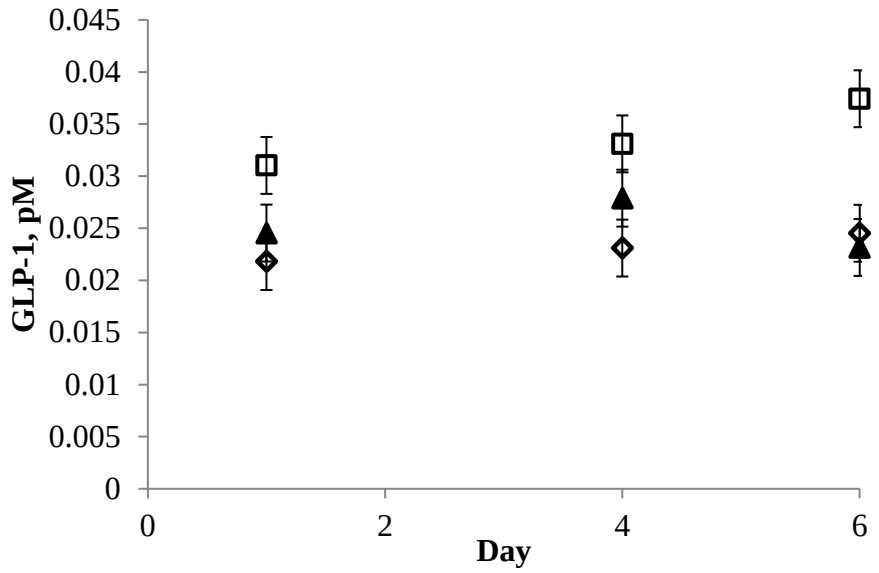


396
397
398 Figure 1

399 Dry matter intake (DMI) over 6 days in wethers fed a diet without supplemental fat (◊) a diet
400 with the addition of 6% Ca salts of palm oil (□) or the control diet and infused with 0.155 µg/kg
401 BW/day of GLP-1 (7-36) amide (▲). Time by treatment interaction ($P < 0.05$).

402

403



404

405 Figure 2

406 Plasma concentration of glucagon like peptide-1 (7-36) amide (GLP-1) over 6 days in wethers

407 fed a diet without supplemental fat (◊) a diet with the addition of 6% Ca salts of palm oil (□) or

408 the control diet and infused with 0.155 µg/kg BW/day of GLP-1 (7-36) amide (▲). Treatment

409 effect ($P < 0.01$), time by treatment interaction ($P = 0.50$).

410