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

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14

15 **ABSTRACT**

16 The objectives of the present study were to determine effects of jugular vein infusions of
17 glucagon like peptide-1 (GLP-1) and dietary fat inclusion on dry matter intake, nutrient
18 digestibility and hypothalamic mRNA concentration of neuropeptide Y, agouti related peptide,
19 and proopiomelanocortin in growing sheep. Thirty six wethers were used (40.7 ± 3.3 kg BW).
20 Treatments were a control diet (n = 11), dietary addition (6% of dry matter) of Ca salts of palm
21 oil fatty acids (n = 12), or 6-d jugular vein infusions of 0.155 µg/kg body weight/day of GLP-1
22 (n = 11). Hormone concentrations were measured in jugular vein plasma from samples taken on
23 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

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

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

50

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

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

53

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

58

59 INTRODUCTION

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

68 reported that ICV infusion of GLP-1 decreased NPY and agouti-related peptide (AgRP) and
69 increased proopiomelanocortin (POMC) mRNA concentration in the hypothalamus of fasted
70 rats. In ruminants, *in vitro* culture of sheep hypothalamus in media containing GLP-1 did not
71 change the relative concentration of NPY, AgRP or POMC mRNA (Relling et al., 2012).
72 However, an increase in NPY and AgRP mRNA was associated with an increase in plasma GLP-
73 1 concentration and a decrease in DMI when fat was fed to growing lambs (Relling et al., 2010).
74 There is a paucity of information on the effect of intravenous infusion of GLP-1 on the mRNA
75 concentration for hypothalamic neuropeptides associated with DMI regulation and its association
76 with DMI. Based on the cited literature, we hypothesized that increases in plasma GLP-1
77 concentration within physiological concentrations, due to continuous jugular vein infusion of
78 GLP-1 or by feeding fat, would decrease DMI. We also hypothesized that decreases in DMI
79 would be associated with changes in hypothalamic gene expression of NPY, AgRP and POMC.
80 Therefore the objective of our study was to determine the effect of a continuous jugular vein
81 infusion of GLP-1 or feeding fat on plasma GLP-1 concentration, DMI, and mRNA
82 concentration of the neuropeptides NPY, AgRP and POMC in growing wethers.
83

84 **MATERIALS AND METHODS**

85 Animal care followed guidelines recommended in the *Guide for the Care and Use of*
86 *Agricultural Animals in Agricultural Research and Teaching* (FASS, 1998) and procedures used
87 were approved by the Animal Care Committee of the Ohio Agricultural Research and
88 Development Center.
89 Three weeks before the start of the experiment, 36 Targhee x Hampshire wethers (40.7 ±
90 3.3 kg BW) were fed a pelleted control diet (Table 1) formulated to meet nutrient requirements

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

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

116 et al., 2011) and started at 1000 h on day 1 of the experimental period. Briefly, GLP-1 solutions were
117 prepared using 1 L of sterile saline solution (9 g/L of NaCl; VWR International, West Chester, PA). The
118 liter of saline solution was infused at a rate of 0.725 ml/min during 23 h. The wethers fed the
119 control and the fat supplemented diets were intravenously infused with 1 L of sterile saline solution (9 g/L
120 of NaCl). The targeted dose of GLP-1 infused was calculated using a single compartment, first-order
121 kinetic hormone degradation model, based on the equation:
122 *Increase in hormone concentration x 0.693/half life.*
123 The half life used for this equation was 5 min for GLP-1 (Perfetti and Merkel, 2000). The
124 value, 0.693, is the slope of the first order degradation. The target increase for GLP-1 was based
125 on a previous report (Relling et al., 2011).
126 The bottles with sterile saline solution and those with GLP-1 in solution were kept on ice
127 during the infusion. The infusion line from the bottle to the animal was sterilized using an
128 ethylene oxide (EtO) gas (Cole-Parmer, Vernon Hills, IL). The connection between the bottle
129 with the infusion and the infusion line included a sterile 0.45 μm syringe filter (Whatman
130 International Ltd, Florham Park, NJ).
131 Between the end of each day's infusion and the start of the following day, the infusion
132 lines were flushed with sterile saline solution (9 g/L of NaCl) and the filters were changed. Feed
133 was offered daily at 1300 h and the refusals were removed and weighed 23 h later at 1200 h. For
134 digestibility and plasma samples, samples were collected and processed as described previously
135 (Relling et al., 2011). Briefly, to measure digestibility, total fecal collection was performed daily
136 during the last 5 d of each experimental period. Five percent of the total daily feces was collected
137 and composited for analysis of DM (100°C oven for 24 h), neutral detergent fibre (NDF,
138 (Ankom^{200} Fiber Analyzer, ANKOM Technology, Fairport, NY), crude protein (CP, Kjeldahl N

139 x 6.25), fatty acids (FA, Sukhija and Palmquist, 1988), and ash (AOAC 1990) concentration.

140 Blood samples (10 ml) were taken 6 and 8 h after feed was offered on day 1, 4, and 6 of each

141 experimental period. Blood samples were immediately transferred into polypropylene tubes

142 containing solutions of disodium EDTA and benzamidine HCl (1.6 mg and 4.7 mg/ml blood,

143 respectively) and placed on ice. After centrifugation for 25 min at 1800 x *g* and 4°C, plasma was

144 partitioned into individual polypropylene tubes for each analysis to be performed, flash frozen

145 using liquid N_2 within 40 min of sample collection, and stored at -80°C until analyzed. Samples

146 from the infusate were taken after the in line filters during the first sampling time on day 4 to

147 confirm that the infusate contained the correct concentration of GLP-1. Measured GLP-1

148 concentrations in the infusate were within 98.2% (±3.7, $P = 0.798$) of targeted concentrations.

149 Concentrations of insulin and GLP-1 were measured using radioimmunoassays as described

150 previously (Reynolds et al., 1989; Benson and Reynolds, 2001). The intra-assay CV averaged

151 less than 12.5% for insulin and less than 11% for GLP-1. Minimum sensitivities (90% of zero

152 standard binding) of the insulin and GLP-1 assays were 0.0027 and 0.001 ng/tube, respectively.

153 Plasma glucose concentration was measured using a colorimetric assay (#1070 Glucose Trinder,

154 Stanbio Laboratory, Boerne, TX). Plasma NEFA concentration was measured using microtiter

155 plates and a plate reader in a two-reaction, enzyme based assay (Wako Chemicals USA,

156 Richmond, VA) as described by Johnson and Peters (1993).

157 The morning of the seventh day of infusions, the lambs were transported 165 km

158 (transport time was 100 min) to an abattoir for hypothalamus collection. It has been previously

159 reported (Relling et al., 2010) that there were no effects of the same transportation routine on the

160 mRNA concentration for the same genes in the hypothalamus of similar lambs (Relling et al.,

161 2010). The hypothalamus was collected within 1 hour after arrival to the slaughter house as

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

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

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

186 The data were statistically analyzed as a complete randomized block design with repeated
187 measurements in time using the MIXED procedure of SAS (Version 9.1, SAS Institute, Cary,
188 NC) and a model testing the random effects of wether and block, and the fixed effect of
189 treatment and time and their interaction. The two daily plasma samples for hormones and
190 metabolites from the three days of sampling in each experimental period were analyzed in the lab
191 individually but the average for each day was used in the statistical analysis. For digestibility and
192 mRNA concentration data, a similar statistical model was used without the effect of time and its
193 interaction. When the time by treatment interaction was significant, the slice option of SAS was
194 used for separation of means. Fisher's protected LSD test was used for means separation at a *P*
195 value of 0.05, for digestibility, mRNA concentration, and when the time by treatment interaction
196 was not significant ($P > 0.10$). Trends were discussed for *P* values between 0.05 and 0.10.
197

198 **RESULTS**

199 There was a time by treatment interaction for DMI ($P < 0.05$; Figure 1), due to a greater
200 DMI for GLP-1 and control-fed wethers compared with the fat-fed wethers on day 1, but no
201 difference in DMI on day 6 for the three treatments. Metabolizable energy intake and
202 digestibility of DM, CP, FA and organic matter (OM) was not different among the treatments (*P*
203 > 0.10; Table 3). The addition of dietary fat decreased ($P < 0.05$) and there was a trend for GLP-
204 1 infusion to increase ($P < 0.10$) NDF digestibility compared with control fed wethers. Feeding
205 fat or GLP-1 infusion did not change plasma concentrations of insulin and glucose ($P > 0.30$;
206 Table 4) compared with the control wethers. Compared with control wethers, plasma GLP-1

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

210 **DISCUSSION**

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

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

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

241 Feeding fat increased plasma GLP-1 concentration, but the infusion of GLP-1 did not
242 change plasma GLP-1 concentration compared with control-fed wethers. As shown in Figure 2,
243 infusion of GLP-1 tended to increase plasma GLP-1 concentration on day 4 but then
244 concentrations decreased on day 6. The lack of response of plasma GLP-1 concentration to GLP-
245 1 infusion could be due to a decrease in endogenous secretion into blood, an increased clearance
246 rate, or both; however, we are not aware of studies that can support this assumption. This lack of
247 response of plasma GLP-1 concentration was unexpected and may in part explain the lack of
248 effects of GLP-1 infusion on DMI. However, this lack of response in the GLP-1 infused wethers
249 does not explain the observed increase in NDF digestibility.

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

253 plasma NEFA occurred concurrently with an increase in plasma GLP-1 concentration. However,
254 infusion of GLP-1 did not increase plasma NEFA concentration. The lack of response on plasma
255 NEFA concentration observed in the present study and observed previously (Relling et al.,
256 2011), suggests GLP-1 infusion does not change plasma NEFA concentration.

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

272

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279

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335 **A.; Herbert, J.; Bloom, S. R. 1996**. A role for glucagon-like peptide-1 in the central
336 regulation of feeding. Nature **379:** 69-72.
337

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

340

341 [z] Intravenous GLP-1infused

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

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

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

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

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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

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

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

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

368

369 Table 4

370 Plasma hormone and metabolite concentration in growing wethers fed a control diet, the control

371 diet plus 6% Ca salts of palm oil (6% Fat), or infused intravenously with 0.155 µg/kg BW/day of

372 GLP-1. Due to lack of time by treatment interaction values represent average of day 1, 4 and 6.

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	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

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377 [z] Trt= treatment effect; TxT = time by treatment interaction effect; GLP-1= glucagon-like

378 peptide-1 (7-36) amide; NEFA= non esterified fatty acid.

379 * Differs from control, $P < 0.05$.

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383 Table 5

384 Hypothalamic concentrations of mRNA in growing wethers fed a control diet, the control diet

385 plus 6% Ca salts of palm oil (6% Fat), or infused intravenously with 0.155 µg/kg BW/day of

386 GLP-1.

387

	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

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390 [z] Concentrations of mRNA (relative to cyclophilin B) for neuropeptide Y (NPY), agouti related

391 peptide (AgRP), and proopiomelanocortin (POMC).

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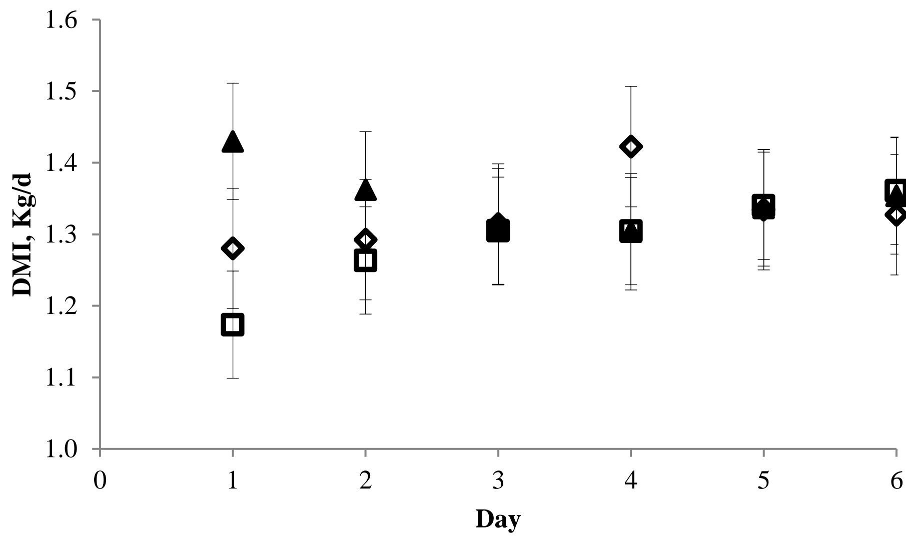

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398 Figure 1

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).

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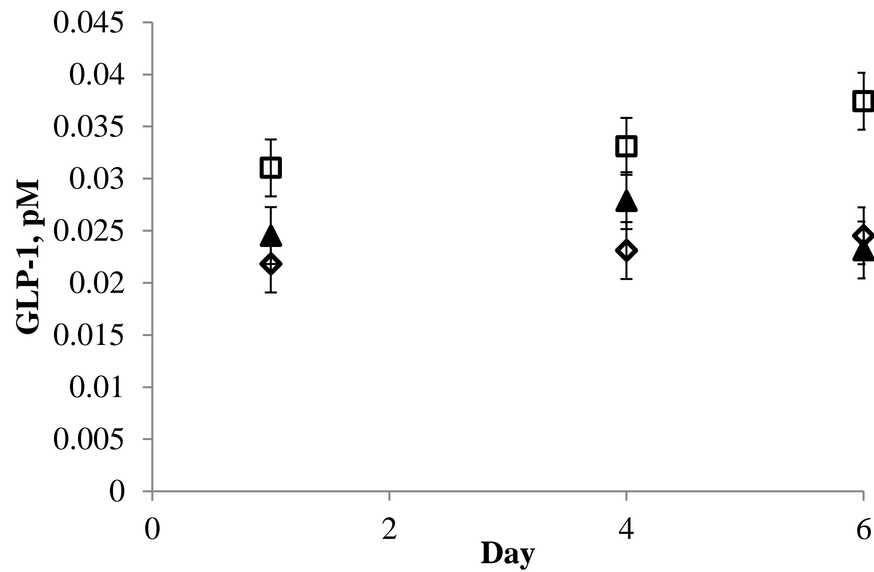

404

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 ($P = 0.50$).

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