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Manipulating Incretin Secretion From The Gastrointestinal Tract To Regulate Feed Intake In Pigs

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Executive Summary

Background:

Incretins are a group of gastrointestinal hormones that can influence glucose metabolism and satiety following a meal. These hormones act to collectively decrease the concentration of circulating plasma glucose. The manipulation of these hormones may have implications for the strategic manipulation of feed intake in pigs, amongst other things. There is limited information on the action of these hormones in pigs, although there is some evidence indicating that this axis is stimulated by glucose absorption in the small intestine. Manipulating incretin secretion following a meal may therefore impact nutrient partitioning via changes in pancreatic glucose sensitivity and insulin release, feed intake, and by altering gastric motility and satiety signals. In pigs, research to date suggests that this effect may be more pronounced when the diet contains higher levels of sugar. One of the two major incretins is glucagon-like peptide-1 (GLP-1) and chronic administration of GLP-1 can result in weight loss in humans and rats. However, GLP-1 is rapidly degraded by circulating dipeptidyl peptidase IV (DPP IV) meaning that an oral administration of GLP-1 is unlikely to have any therapeutic benefits. Thus, the use of orally active DPP IV inhibiting compounds provide an opportunity to manipulate the secretion of incretin hormones, which have the potential to be incorporated into commercial pig production systems.

Objectives/hypothesis:
An example of a DPP IV inhibitor is sitagliptin, which is currently marketed as a human diabetic drug. This study was designed to explore the effects of the orally active DPP IV inhibitor sitagliptin on feed intake and nutrient partitioning in growing pigs. As DPP IV inhibition promotes the secretion of incretins, it is hypothesised that the addition of sitagliptin will decrease feed consumption in pigs. Furthermore, when fed in combination with a diet containing added sugar (25%) it is hypothesised that sitagliptin supplementation would further increase incretin actions as the presence of glucose would further stimulate their secretion from the gastrointestinal tract.

Key findings:

- For the first two weeks of feeding there was no difference in Feed conversion ratio (FCR) or intake due to diet or treatment. Feed intake was greater for pigs receiving the sugar diet in weeks 3 (+ 2.34 kg, P=0.021) and 5 (+ 1.69, P=0.052) and FCR tended to be greater in week 4 (+ 1.52 kg, P=0.072).
- The FCR was not influenced by sitagliptin treatment for the duration of the experiment.
- Pigs receiving the sugar diet had a higher (+0.17) FCR than those receiving control diets at week 2 (P=0.05); which tended to remain true at week 3 (+0.13, P=0.083).
- Plasma glucose concentrations were not influenced by the sugar diet or sitagliptin in the pre feeding period. However, there was an interaction (P=0.005) between sugar and sitagliptin such that pre feeding glucose concentrations were lower in pigs fed the sugar diet compared to control and was decreased by increasing sitagliptin dose in pigs receiving the control diet. The reverse of this relationship occurred in those receiving the sugar diet and glucose concentrations increased with increasing sitagliptin dose.
- In the post feeding period there was an effect of time (P=0.03) whereby the greatest plasma glucose concentration was achieved 30 mins and the lowest at 180 mins post feeding.
- The sugar diet did not influence plasma glucose concentrations in the post feeding period.
- There was an interaction between diet and treatment (P=0.009) such that plasma glucose concentration decreased with increasing doses of sitagliptin in pigs fed the control diet, while the reverse occurred in those fed sugar and plasma glucose concentrations increased with the 100mg sitagliptin dose.

- Plasma IGF-1 ($P=0.05$) and insulin ($P<0.001$) concentrations were increased in post feeding, but were not influenced by diet type (sugar or control) or sitagliptin dose.
- There was an effect of sugar ($P<0.01$) on carcass DXA measurements such that BMC increased, fat % increased and carcass lean % was decreased in pigs receiving the sugar compared to the control diet.
- Sitagliptin had no effect on carcass characteristics.

Conclusions and future research:

This project identified that stimulation of the incretin axis via the use of the DPP IV inhibitor sitagliptin can successfully reduce feed intake without altering FCR; suggesting that intake is reduced while weight gain remains stable. However, this effect waned as the study progressed, perhaps indicating that the dose of sitagliptin needs to be matched to live weight in order to elicit a detectable response. There was no effect of sitagliptin on plasma hormone concentrations (insulin and IGF-1) or carcass characteristics, which is also likely due to the wane in response seen as the pigs grew. This was a novel finding and to our knowledge there are no published studies examining the long term effects of DPP IV inhibitors in pigs, but is a promising finding as it suggests that orally active DPP IV inhibitors have the potential to be used to modify intake in a commercial environment. Furthermore, there was an unexpected response whereby plasma glucose concentrations were influenced by both sitagliptin and sugar in both the pre and post feeding measurements. Plasma glucose concentrations were decreased by increasing sitagliptin dose in pigs fed the sugar diet while the reverse was true in those fed the control diet and concentrations increased with increasing sitagliptin dose, suggesting that in the pigs fed the high sugar diet sitagliptin is able to increase incretin activity. This response has not been previously demonstrated in pigs or any other species. The higher glucose concentration in the diet with added sugar was hypothesised to enhance insulin release from the pancreas and hence increase the rate of glucose clearance following a meal, although this was not reflected by differences in plasma insulin concentrations. The decreased glucose concentration with increasing sitagliptin concentration post feeding in pigs fed the sugar diet suggests that there is an increased insulin secretion in response to glucose that is resulting in a decrease to plasma glucose concentrations, although this was not reflected by measures of plasma insulin in this experiment. It is suggested that perhaps sitagliptin is causing a delay in gastric emptying, although this cannot be elucidated from the current experiment. Further research is required to determine the responses underpinning the changes in glucose concentration measured here. Additionally, further studies examining doses of sitagliptin that are fed on a live weight basis would be beneficial, as it is suggested that higher doses of sitagliptin will maintain the reduction in feed intake noted in the initial period of this study. Finally, additional metabolic studies such as glucose challenges, measures of gastric emptying and sequential measurements of fat and muscle depths (using DXA etc.) combined with a dose of sitagliptin based on live weight are recommended to further evaluate the relationships demonstrated in the current study.

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1. Background to Research

Under the "Nutrition and Physiology" portfolio APL have identified that two key areas are "Manipulating feed intake" and "Finisher pig growth promoting technologies" and this project was designed to contribute to both of these areas. The discovery of gastrointestinal hormones that can influence glucose metabolism and satiety following increases in glucose (and lipid in some species) absorption following a meal could have implications for the strategic manipulation of feed intake in pigs. Collectively these hormones were termed '*incretins*' because they increased the sensitivity of the pancreas to blood glucose concentration, resulting in a lowering of blood glucose. There is very little data on the importance of the incretin axis in pigs, although there is some data to indicate that this axis in pigs is stimulated by glucose absorption in the small intestine (Drucker, 2006). Thus, manipulating incretin secretion following a meal can potentially impact nutrient partitioning, through changes in pancreatic glucose sensitivity and insulin release, and feed intake, through changes in gastric motility and satiety signals. In the pig, this effect may be more pronounced if the diet contains higher levels of sugar, because research to date indicates that the effectiveness of DPP IV inhibitors is only evident when blood glucose concentration is elevated. Understanding the regulation of the incretin axis in the pig could provide important information on mechanisms for strategically manipulating feed intake in the pig using dietary means and/or orally active agents that alter incretin activity. This data will also enhance the likelihood of developing links with emerging commercial partners that are actively engaged in discovery and/or development of naturally derived compounds with DPP IV inhibitory activity, and possibly GLP-1 agonists. Currently, there is no obvious commercial partner, other than the pharmaceutical companies that manufacture a range of DPP IV inhibitors.

2. Objectives of the Research Project

This project aims to confirm that the incretin axis plays an important role in the regulation of feed intake, growth rate and nutrient partitioning in finisher pigs as the animal model. It also aims to establish whether the orally active DPP IV inhibitors and specific nutrients, such as dietary glucose, can stimulate the incretin axis in the finisher pig. This information can used by nutritionists and feed compounders to develop strategies to manipulate feed intake at key stages of the production system. This data will also enhance the likelihood of developing links with emerging commercial partners that are actively engaged in developing naturally derived compounds with DPP IV inhibitory activity.

1. To confirm that the incretin axis is important for the regulation of feed intake in pigs

2. To confirm that orally active DPP IV inhibitors stimulate the activity of incretins resulting in a reduction in feed intake in pigs

3. To determine whether dietary glucose stimulates the incretin axis in pigs

3. Introductory Technical Information

The discovery of hormones derived from the gastro intestinal tract (GIT) occurred after noting that the secretion of insulin was greater when glucose was ingested orally compared to a similar glucose load delivered intravenously (Wideman and Kieffer, 2009). Incretin hormones are gut derived factors secreted in response to nutrient detection in the intestine and act to enhance the actions of insulin by increasing secretion from pancreatic β cells (Drucker, 2006). Incretins are estimated to be responsible for 70% of post-parandial β -cell activity (Baggio and Drucker, 2007). The two major incretins are glucose-dependent insulinotropic polypeptide (GIP, previously gastric inhibitory polypeptide) and glucagon-like peptide-1 (GLP-1) (Deacon, 2005; Wideman and Kieffer, 2009). Receptors for GLP-1 and GIP are found in various tissues, including β -cells, and insulin gene transcription is promoted by GLP-1 (Drucker et al., 1987). In addition to the pancreas, GLP-1 receptors are found in the gut, kidney, heart, muscle and lung tissues (Sivertsen et al., 2012). Both GIP and GLP-1 have been shown to affect a variety of mechanisms related to ingestion and feeding behaviour in monogastric species. Secretion of these two hormones from the GIT is stimulated when glucose absorption in the small intestine increases following carbohydrate digestion which leads to a post-prandial increase in blood glucose (Drucker, 2006). These hormones potentiate glucose-stimulated pancreatic insulin secretion and decrease glucose production in the liver (Deacon, 2005; Seino et al., 2010; Wideman and Kieffer, 2009).

The physiological role of GLP-1 in the regulation of glucose has been previously reviewed (Baggio and Drucker, 2007; Drucker, 2006) and will not be covered here. Importantly, GLP-1 can inhibit gastric emptying and therefore increase satiety and in turn decrease feed intake (Wideman and Kieffer, 2009). The role of GLP-1 in appetite and body weight has been extensively reviewed by van Bloemendaal et al. (2014) and will not be discussed in depth here, although it is worth noting that these responses are due to the combination of effects on gastric distension, peripheral vagal nerve activation, induction of satiety and acting upon the 'hunger centres' in the brain. Both GIP and GLP-1 primarily respond to the ingestion of carbohydrates and fats, but they also respond to a lesser degree to the absorption of proteins or amino acids (Brubaker, 2006). Interestingly, GLP-1 receptor concentrations increase in the small intestine with normal development and these changes can be manipulated by dietary changes during the neonatal and weaning periods (Van Ginneken et al., 2002). A meta-analysis of trials involving the use of GLP-1 agonists in humans demonstrated that all trials resulted in weight loss with losses ranging from -7.2 to -0.2 kg (Vilsbøll et al., 2012). Interestingly, GLP-1 agonists were also able to reduce

systolic and diastolic blood pressure as well as total cholesterol (Vilsbøll et al., 2012). There has been increased recent investigation into the effects of GLP-1 agonists on cardiovascular health in humans, as reviewed by Sivertsen et al. (2012).

Chronic administration of GLP-1 can lead to weight loss in humans (Field et al., 2008; Flint et al., 1998; Horowitz et al., 2012) and rats (Tang-Christensen et al., 1996; Turton et al., 1996) by influencing the central nervous system (CNS), triggering a reduction in appetite and subsequently intake (Tang-Christensen et al., 1996; Turton et al., 1996; van Bloemendaal et al., 2014). In addition, studies have suggested that GLP-1 action in the brain promotes a reduction in insulin-stimulated glucose uptake in muscle and favours enhanced liver glycogen storage, signals communicated via neural pathways (Knauf et al., 2005). However, as a peptide GLP-1 is rapidly degraded by circulating dipeptidyl peptidase IV (DPP IV) meaning that an oral administration of GLP-1 is unlikely to have any therapeutic benefits. DPP IV inhibitors on the other hand enhance the endogenous levels of incretins and are commonly used in the treatment of diabetes. As DPP IV rapidly and completely inactivates GLP-1 (Field et al., 2008), DPP IV inhibitors act to prevent this occurring. DPP IV is present in cells within the cell membrane and is also present in circulation (Faidley et al., 2006).

Enhancing the activity of incretins to reduce high blood glucose concentrations has been the subject of considerable recent research as a means to treat Type II diabetes (Brubaker, 2007; Davis et al., 2010; Hoist and McGill, 2012; Nisal et al., 2012; Poucher et al., 2012; Waget et al., 2011; Wideman and Kieffer, 2009). Incretin mimemtics and agonists (primarily for GLP-1) are being developed, but to date these have to be injected and this may pose some issues for long term use in animals. However, they have the advantage of having specific functions, including weight loss, depending on dose used. In addition, one primary mechanism that limits incretin hormone activity is inactivation by the enzyme Dipeptidyl Peptidase-4 (DPP IV), which is found throughout the body. This occurs rapidly and inactivates both GLP-1 and GIP (D'Alessio, 2011; Deacon et al., 2001; Faidley et al., 2006; Ohlsson et al., 2013). Several DPP IV inhibitors have been developed to increase the activity of naturally occurring incretins i.e. to lower blood glucose, and are available for use in humans, such as sitagliptin (Wideman and Kieffer, 2009). The advantage of using DPP IV inhibitors is that they are orally active and could be incorporated into feed. Moreover, while this project aims to utilize a synthetic DPP IV inhibitor, which is the only type available at present, there is active research ongoing to identify naturally occurring compounds with DPP IV inhibitory activity. The most promising are some specific dairy protein hydrolysates which have shown DPP IV inhibition under in vitro conditions (Lacroix and Li-Chan, 2012), indicating the potential for naturally derived compounds to be available in the future. In addition, there are examples of other specific nutrients (including cinnamon) that might be linked to the regulation of the incretin axis (Hlebowicz et al., 2009).

An acute dose of the DPP IV inhibitor sitagliptin (100 mg) increased GLP-1 concentrations in healthy (non-diabetic) human subjects following ingestion of equicaloric either glucose, olive oil or protein (milk and egg protein) (Ohlsson et al., 2013). This response was greater following the ingestion of fat (oil) and also resulted in a significantly lower glucose response (as measured by area under the curve, AUC) in subjects treated with sitagliptin (Ohlsson et al., 2013). Interestingly, Ohlsson *et al* (2013) also demonstrated that there was no effect of sitagliptin on insulin responses to any of the diets consumed, indicating that there was an increased sensitivity to glucose without influencing the actions of insulin producing β -cells. It is therefore suggested that sitagliptin lowers glucose concentrations by delaying gastric emptying and reducing glucose absorption.

In pigs (n = 10), DPP IV inhibition via a sitagliptin analogue for 72 hours did not alter the growth hormone (GH) and insulin-like growth factor-1 (IGF-1) axis (Faidley et al., 2006). It has also been

shown that DPP IV inhibition (using valine-pyrrolidie) in pigs reduces the clearance of GIP and increases the levels of intact and biologically active GIP (Deacon et al., 2001). DPP IV inhibition combined with GIP infusion and followed by a glucose load also resulted in an increased plasma insulin concentration before and after the glucose infusion, while glucose clearance was also increased at a more rapid rate in the presence of the DPP IV inhibitor (Deacon et al., 2001). While the study by Deacon *et al* (2001) was in a small number of pigs and did not measure the long-term effects of DPP IV inhibition, these results demonstrate that manipulation of the incretion hormones is possible and can be used to manipulate glucose homeostasis in pigs. This is promising as it suggests that supplementation of DPP IV inhibitors may be a successful method for manipulating intake and weight gain in commercial pigs, although further research is required to full elucidate the potential of these treatments.

4. Research Methodology

This study was conducted at The University of Melbourne, Dookie Campus, piggery facility for 8 weeks. All procedures were approved by The University of Melbourne Animal Ethics Committee. A total of 36 female finisher pigs (average weight approximately 66.8 kg ± 0.84 kg and 3 months old) were weighed and randomly allocated based on stratified live weight to 6 treatment groups. All pigs had *ad libitum* access to water and feed for the entire experimental period. The experimental diets were formulated to meet the nutrient requirements of pigs according to the National Research Council guidelines (1998). Treatment groups (n=6 per group) contained a finisher diet with:

1. Nil DPP IV inhibitor (control)
2. Low (25 mg/d) DPP IV inhibitor (sitagliptin, Merck Co.)
3. High (100 mg/d) DPP IV inhibitor
4. 25% sugar with nil DPP IV inhibitor
5. 25% sugar with low DPP IV inhibitor
6. 25% sugar with high DPP IV inhibitor

4.1 Measurements

Feed intake and weight gain were recorded weekly for 8 weeks. Pigs were weighed by standard calibrated electronic scales. Feed was offered *ad libitum* on a daily basis such that there was 10 -15 % residue daily. At the end of each week total residues were weighed and average feed intake calculated. At 0800 h at the start of week 7 all pigs were fitted with an ear vein catheter to allow the collection of multiple blood samples. Briefly, pigs were restrained using a snout rope and the ear cleaned with Betadine antiseptic solution and 70% ethanol. A suitable ear vein was located and an 18g x 1¼ inch I.V. catheter (Surflo I.V. catheter, Terumo Corp) inserted. A Teflon wire guide (0.81 mm diameter, 90 cm length, Radifocus Glidewire Terumo Corp. Australia) was then passed through the catheter into the vein for approximately 30 cm. A single lumen polyethylene catheter (0.97mm ID X 1. 27mm ID, 120cm in length, Tyco Electronics Pty Ltd, Australia) was passed over the wire guide and into the ear vein to a depth of approximately 35 cm, placing the catheter tip in an external jugular vein. The wire guide was then removed and the catheter flushed with heparinised saline (150 IU/ml) and plugged with a sterile sampling port. The site was secured using Elastoplast tape and the catheter was wrapped in 7.5cm wide elastic adhesive tape (Elastoplast) and taped to the ear. The ear was then taped to the body of the pig with Elastoplast and the catheter secured within a pouch covered with Elastoplast on the dorsal midline of the pig. Pigs were given at least 60 mins to settle post insertion of the catheter prior to any blood samples being collected.

Pigs were fasted for 12 hrs (overnight) and blood samples obtained via the ear vein catheter in the pre/post parandial periods at -45, -30, -15, 15, 30, 45, 60, 90, 120 and 180 mins. Blood samples were centrifuged at 3000rpm for 10 mins at 4°C then frozen at -20°C until further analysis. Plasma was analysed for key metabolites Plasma glucose concentrations were measured using a Glucose oxidase kit (Thermo Scientific). The insulin assay utilized a double-antibody radioimmunoassay method of Hales and Randle (1963) as modified by Bassett and Wallace (1996) and described by Tindal *et al* (1978). Insulin-like growth factor-1 (IGF-1) was measured using the chloramine-T radioimmunoassay method described by Gluckman *et al* (1983). Samples were measured in duplicate, and interference by binding proteins was minimised using the acid-ethanol cryoprecipitation method validated for ruminants by Breier *et al* (1991) . The intra-assay coefficients of variation were 5.1% and 8.6% and the limit of detection was 0.5 ng/ml. Following the collection of the pre/post parandial blood samples all pigs were slaughtered using lethabarb (0.5 ml/kg). The whole carcass was retained and scanned onsite using dual energy x-ray absorptiometry (DXA) to measure total body carcass composition. A Hologic Discovery W Fan Beam X-Ray Bone Densitomer (Hologic Inc., Waltham, MA, USA) was used to determine body

composition in entire pigs post-slaughter. The whole-body scan mode was used (software version 8.26a:3). Measurements included total tissue mass (TTM), lean tissue mass (LTM), fat tissue mass (FTM) and bone mineral content (BMC).

4.2 Statistical Analysis

Intake, live weight and feed conversion ratio (FCR) responses are reported as mean values unless otherwise stated. Statistical analysis was conducted using the ANOVA function in GenStat 15th edition (Payne et al., 2008). Responses were also analysed for any linear relationships due to sitagliptin dose. Data was blocked for location within the shed and the main factors were diet (control or sugar) and sitagliptin concentration (0, 25 or 100 mg/day) and all possible interactions explored. Plasma glucose responses in the pre and post parandial periods were analysed for the effects of diet and treatment and mean values reported.

5. Results

Intake and feed conversion responses to the dietary treatments are summarised in Table 5.1. Feed intake did not differ due sitagliptin (treatment) dose for the duration of the experiment. Feed intake was greater for pigs receiving the sugar diet in weeks 3 (+ 2.34 kg, P= 0.021) and 5 (+ 1.69, P= 0.05) and tended to be greater in week 4 (+ 1.52 kg, P = 0.07). There was a trend for diet and treatment to interact in week 4 (P=0.086) such that intakes were lowest in the control diet while sitagliptin increased feed intake in the pigs fed the sugar diet and decreased intake in pigs fed the control diet. Feed conversion ratio (FCR) was not influenced by sitagliptin treatment for the duration of the experiment. Pigs receiving the sugar diet had a higher FCR than those receiving control diets at week 2 (2.36 vs. 2.53 for control and sugar respectively, sed 0.0844, P=0.05); which tended to remain true at week 3 (2.69 vs. 2.82 for control and sugar respectively, sed 0.0687, P=0.08).

Plasma glucose concentrations in the pre and post feeding periods are presented in Tables 5.2 and 5.3 respectively. In the pre feeding period there was no effect of the either the sugar diet (P=0.33) or sitagliptin (P=0.14) on plasma glucose concentrations. There was however an (P=0.005) interaction between sugar and sitagliptin such that pre feeding glucose concentrations were lower in pigs fed the sugar diet compared to control and was decreased by increasing sitagliptin dose in pigs receiving the control diet, while the reverse occurred in those receiving the sugar diet and glucose concentrations increased with increasing sitagliptin dose. Glucose concentrations were trending towards being reduced by sitagliptin regardless of dose (4.96, 4.57 and 4.59 mM for 0, 25 and 100mg sitagliptin respectively, sed 0.1894, P=0.08). In the post feeding period there was an effect of time whereby the greatest plasma glucose concentration (4.98 mM) was achieved at 30 mins post feeding and the lowest (4.51mM) measured at 180 mins post feeding (4.98, 4.65, 4.68, 4.70, 4.51 mM for 30, 60, 90, 120 and 180 mins respectively, sed 0.148, P=0.03). The sugar diet did not influence plasma glucose concentrations in the post feeding period (P= 0.42). There was an interaction between diet and treatment (sed 0.267, P=0.009) such that plasma glucose concentration decreased with increasing doses of sitagliptin in pigs fed the control diet (5.35, 4.63 and 4.33 mM for 0, 25 and 100 mg respectively) while the reverse occurred in those fed sugar and plasma glucose concentrations increased with the 100mg sitagliptin dose (4.58, 4.51 and 4.84 mM for for 0, 25 and 100 mg respectively). There were no higher order interactions between diet, treatment and time influencing post feeding plasma glucose concentrations (Table 5.3).

In the pre feeding period there was a linear effect of sitagliptin dose on plasma glucose concentrations (sed =0.145, P=0.016) such that pre-feeding glucose concentrations were greatest with 25mg sitagliptin (4.64 mM) and control (4.60 mM) treatments and lower in pigs fed 100mg (4.30mM). In the post-feeding period there was a significant linear relationship (sed 0.186, P=0.004) the control pigs had the highest plasma glucose concentrations (4.96 mM) while the glucose concentrations in the 25mg (4.57 mM) and 100mg (4.59 mM) doses were reduced.

Carcass characteristics as measured by DXA are presented in Table 5.4. There was a significant (Table 5.4, P<0.01) main effect of diet type such that the inclusion of sugar increased bone mineral content (BMC) (1.49 Vs 1.83 % for control and sugar respectively, sed 0.054) and fat % (20.3 Vs 22.1 % for control and sugar respectively, sed 1.132) and decreased lean % (78.2 Vs 76.1 % for control and sugar respectively, sed 1.147). Sitagliptin had no effect on carcass characteristics, and there were no significant interactions between diet and treatment.

Plasma IGF-1 and insulin concentrations measured during the pre- and post-parandial period are presented in Table 5. Plasma IGF-1 concentrations differed with time (P=0.046) such that concentrations reduced post feeding (67.4, 68.4, 68.3, 68.6, 63.1, 64.2, 65.7 and 63.5 ng/mL for -60, -

30, -1, 30, 60, 90, 120 and 180 mins relative to feeding respectively, sed 2.26). However, there was no overall effect of diet type (63.8 Vs 68.5 ng/mL for control and sugar diets respectively, sed 5.02, P = 0.35) or sitagliptin dose (67.6, 62.3 and 68.5 ng/mL for 0, 25 and 100 g/day respectively, sed 6.15, P = 0.55) on plasma IGF-1 concentrations. There were no interactions between any of the variables measured that significantly altered IGF-1 plasma concentrations (Table 5.5).

Plasma insulin concentrations differed with time (P < 0.001) such that concentrations were increased post feeding (5.1, 5.9, 5.8, 20, 23.3, 17.8, 14.6 and 13.0 µU/mL for -60, -30, -1, 30, 60, 90, 120 and 180 mins relative to feeding respectively, sed 1.26). There was no difference in plasma insulin concentrations due to diet type (13.1 vs 13.3 µU/mL for control and sugar diets respectively, sed 0.789, P = 0.83) or due to sitagliptin supplementation (13.4, 13.0 and 13.3 µU/mL for 0, 25 and 100 g/day respectively, sed 0.967, P = 0.89). In addition, there were no interactive effects of any of the factors measured to influence plasma insulin concentrations (Table 5.5).

Table 5.1. Intake and feed conversion ratio (FCR) responses to the DPP IV inhibitor sitagliptin fed to pigs receiving either a control diet or a diet containing added sugar (25%).

	Control			Sugar (25%)				P-values		
Sitagliptin (mg/day)	0	25	100	0	25	100	sed	Diet	Treatment	Diet x treatment
Feed intake (kg)										
Week 1	19.57	19.41	18.62	18.92	19.25	18.19	0.711	0.325	0.147	0.889
Week 2	28.04	26.33	23.52	28.27	28.10	26.57	2.034	0.165	0.106	0.623
Week 3	27.66	25.54	24.54	27.44	28.71	28.61	1.642	0.021	0.703	0.171
Week 4	26.93	25.61	26.19	25.78	28.38	29.13	1.403	0.072	0.432	0.086
Week 5	25.45	24.10	26.13	25.78	26.92	28.05	1.433	0.052	0.237	0.472
Week 6	27.67	27.40	29.03	26.74	29.35	28.96	1.505	0.719	0.253	0.393
FCR										
Week 2	2.38	2.29	2.41	2.56	2.51	2.53	0.146	0.053	0.729	0.862
Week 3	2.79	2.58	2.71	2.82	2.87	2.77	0.119	0.083	0.589	0.243
Week 4	3.15	2.83	3.02	3.09	3.03	2.99	0.138	0.665	0.184	0.341
Week 5	3.35	3.07	3.11	3.23	3.14	3.22	0.122	0.843	0.110	0.368
Week 6	3.71	3.48	3.47	3.65	3.49	3.53	0.163	0.961	0.188	0.870

Table 5.2. Mean pre feeding plasma glucose (mM) concentrations in pigs fed the DPP IV inhibitor sitagliptin (0, 25 or 100mg) and either a control diet or a diet containing added sugar (25%).

				P-values		
Sitagliptin (mg/day)	Control	Sugar (25%)	SED	Sitagliptin	Sugar	Sit x Sugar
0	4.910	4.303	0.2633	0.142	0.333	0.005
25	4.896	4.405				
100	3.989	4.613				

Table 5.3. Post feeding plasma glucose (mM) concentrations in pigs fed the DPP IV inhibitor sitagliptin (0, 25 or 100mg) and either a control diet or a diet containing added sugar (25%).

	Control			Sugar (25%)				P-values						
Time (relative to feeding)	0	25	100	0	25	100	SED	Sitagliptin	Sugar	Time	Sit x Sugar	Sugar x time	Sit x time	Sit x Sugar x time
30 mins	5.361	5.023	4.507	4.818	4.888	5.305	0.4126	0.080	0.422	0.032	0.009	0.759	0.138	0.637
60 mins	4.961	4.480	4.714	4.438	4.378	4.953								
90 mins	5.229	4.768	4.516	4.436	4.479	4.657								
120 mins	5.769	4.382	3.966	4.533	4.529	5.011								
180 mins	5.429	4.513	3.926	4.666	4.256	4.294								

Table 5.4. Carcass dual energy x-ray absorptiometry (DXA) measurements in pigs fed the DPP IV inhibitor sitagliptin (0, 25 or 100mg) and either a control diet or a diet containing added sugar (25%). BMC = bone mineral content.

	Control			Sugar (25%)				P-values		
Sitagliptin (mg/day)	0	25	100	0	25	100	sed	Diet	Treatment	Diet x treatment
BMC %	1.51	1.51	1.47	1.82	1.82	1.86	0.054	<0.001	0.995	0.553
Fat %	21.1	20.0	19.9	21.8	22.6	21.8	1.13	0.012	0.737	0.495
Lean %	77.4	78.5	78.6	76.3	75.5	76.3	1.15	0.004	0.740	0.500
Water	53.4	55.0	54.5	52.4	53.4	53.7	1.68	0.242	0.471	0.944

Table 5.5. Plasma insulin like growth factor-1 (IGF-1) and insulin concentrations in pigs fed the DPP IV inhibitor sitagliptin (0, 25 or 100mg) and either a control diet or a diet containing added sugar (25%).

	Control			Sugar (25%)				P-values		
Sitagliptin (mg/day)	0	25	100	0	25	100	sed	Diet	Treatment	Diet x treatment
IGF-1 (ng/mL)	62.77	60.95	67.72	72.42	63.69	69.33	8.691	0.354	0.549	0.770
Insulin (μU/mL)	12.96	12.90	13.52	13.75	12.99	13.00	1.367	0.831	0.890	0.794

6. Discussion

The results presented here partially support the hypothesis that manipulation of incretion hormones by inhibiting the actions of DPP IV can successfully reduce feed intake without altering FCR in pigs fed a control diet; suggesting that intake is reduced while weight gain remains stable. Therefore, DPP IV inhibition can influence incretin pathways in pigs and this could perhaps be used to manipulate intake in a commercial environment. However, this response waned as the experiment progressed. This is likely due to the rapid increase in live weight of the pigs that was not matched by a concurrent increase in sitagliptin dose. The inclusion of sugar in the diets was designed to further stimulate the incretion pathway by increasing the glucose uptake in the small intestine, and the increased fatness measured in pigs receiving the sugar diet supports the findings of previous studies where pigs fed an *ad libitum* diet containing added sucrose (50%) had a significantly higher fat mass than those fed a control diet (Sabin et al., 2011). However, for reasons that remain unclear, the inclusion of sugar combined with sitagliptin in the diet tended to result in an increase in feed intake, contradicting the hypothesis that DPP IV inhibitors will decrease feed intake. As there was a significant relationship between diet (sugar) and treatment (sitagliptin) to alter plasma glucose concentrations in the pre and post feeding period, this enhanced effect due to sugar is confirmed. There was however an unexpected response seen in the glucose concentration both pre- and post-feeding due to this interaction between sugar and sitagliptin. Intriguingly, plasma glucose concentrations were decreased by increasing sitagliptin in pigs fed the sugar diet while the reverse was true in those fed the control diet and concentrations increased with increasing sitagliptin dose. This response has not been previously demonstrated in pigs or any other species. The higher glucose concentration in the diet with added sugar was hypothesised to enhance insulin release from the pancreas and hence increase the rate of glucose clearance following a meal. This is supported by the post feeding glucose concentrations in the pigs fed sugar, as the decreased glucose concentration with increasing sitagliptin concentration suggests that there is an increased insulin secretion in response to glucose that is resulting in a decrease to plasma glucose concentrations.

However, plasma insulin concentrations were not influenced by sitagliptin when measured in the pre- and post-parandial periods in the final week of the current experiment, which is likely due to the dose of sitagliptin being insufficient to elicit an effect in higher weight pigs. Nevertheless, this does not explain the change in plasma glucose concentrations detected in the present study. In non-diabetic humans, a single dose of sitagliptin (100mg) attenuated the rise in plasma glucose following oral glucose ingestion; although similar to the current study plasma insulin and glucagon concentrations were not influenced by sitagliptin in this study (Ohlsson et al., 2013). Therefore, the actions of sitagliptin may function independent of changes to plasma insulin concentrations. One potential method via which DPP IV inhibitors may influence glucose uptake could involve gastric emptying. In human subjects, the DPP IV inhibitor liraglutide reduced gastric emptying while also reducing meal duration in response to *ad libitum* diets, although no changes in plasma ghrelin or GIP concentrations were detected (Horowitz et al., 2012). However, in the study by Horowitz *et al* (2012), peptide YY (PYY) (an intake regulating peptide secreted by the ileum and the colon in response to feeding) was reduced in patients treated with liraglutide, acting to inhibit gastric motility and suppress pancreatic secretion. Therefore, the reduced plasma glucose concentration noted in the present study may be due to a delay in gastric emptying; and although gastric emptying was not directly measured in the present experiment, this mechanism is supported by the noted reduction feed intake in control pigs supplemented with sitagliptin. However, others have demonstrated no change in gastric emptying due to the supplementation of sitagliptin in human subjects (Ohlsson et al., 2013). A reduction in gastric emptying would delay the progression of physiological signals that 'order the next meal', leading to a reduction in appetite and potentially an increase in nutrient absorption due to the increased meal retention time.

The major incretion GLP-1 is estimated to be responsible for 70% of the β -cell activation stimulated following a meal, and therefore an injection of GLP-1 into the bloodstream results in enhanced insulin secretion and an improvement in glucose disposal (D'Alessio, 2011). On the other hand, DPP IV inhibition is unable to elicit such dramatic increases in GLP-1 due to the limited amount of the peptide that is able to be secreted by the gut (Bock et al., 2010). A suggested, but currently unproven, method via which GLP-1 elicits is actions involves the stimulation of the portal vein which contains GLP-1 specific receptors, with injection of GLP-1 or its agonists directly into the portal vein influencing glucose metabolism (Vahl et al., 2007). Interestingly, oral administration of sitagliptin in a dose insufficient to elicit a change in plasma DPP IV concentrations elicits an inhibition of DPP IV in gut tissues accompanied by an improved tolerance to glucose in wild type mice and not in those without a GLP-1 receptor (Waget et al., 2011). This supports the findings of the present study and although both gut tissue and circulating DPP IV concentrations were not measured, it appears that doses of sitagliptin that are not high enough to produce a change in plasma hormone concentrations are able to influence glucose metabolism.

No changes in plasma IGF-1 concentration were detected in response to sitagliptin supplementation in the current study, which supports the findings of Faidley et al. (2006) who demonstrated no change in GH or IGF-1 concentration in response to DPP IV inhibition via a sitagliptin analogue for 72 hours in pigs. In pigs an increased concentration of IGF-1 results in enhanced growth performance and a reduction in fat depths. Thus, it was hypothesised that a sitagliptin induced increase in FCR and a reduction in fat depth would be partially elicited by an increase in circulating IGF-1 concentrations, which is not supported by the findings of the present study although there was also no effect of sitagliptin on fat depths as measured by DXA. Faidley *et al* (2006) suggest that DPP IV inhibition is therefore not suited for use as a growth promoter, although the initial positive effects on FCR noted in the present experiment suggest that DPP IV inhibitors are suited as a means of carcase modification and improved efficiency in pigs, although further research is required to confirm these relationships.

7. Implications & Recommendations

While the final results of this study only partially support the hypothesis that orally active DPP IV inhibitors can manipulate intake and carcase characteristics in pigs, there were some positive findings that highlight some key areas for future research. Firstly, due to the rapid growth of pigs the dose of sitagliptin (or other DPP IV inhibitors) should be increased in an incremental manner or matched to live weight as the noted effects (in terms of intake) appear to wane in response to the growth of the pigs. Higher doses of sitagliptin may also result in a greater response in pigs and therefore multiple doses, as well as a dose matched to live weight, warrant further exploration. Furthermore, frequent measurement of body composition (e.g. via DXA) throughout growth in pigs fed DPP IV inhibitors (at an appropriate dose) may highlight any changes in adipose tissue as well as gastrointestinal tract weights that are occurring in response to the increased incretin hormone secretion induced. In addition, future studies exploring the effect of DPP IV inhibitors on gastric emptying and PYY secretion in pigs are warranted, as the results from the current study as well as those published elsewhere suggest that gastric emptying is delayed via the actions of DPP IV inhibitors (Horowitz et al., 2012). Similarly, the collection of gut tissue combined with frequent blood samples from pigs supplemented with DPP IV inhibitors would be beneficial for the measurement of circulating DPP IV concentrations as it is suggested that there is not a direct relationship between gut and circulating DPP IV concentrations (Waget et al., 2011). Finally, the measurement of portal vein blood in pigs supplemented with oral DPP IV inhibitors would further discern any relationship between an increase in GLP-1 concentration (produced via DPP IV inhibition) and glucose disposal, which is suggested to be driven by the actions of the portal vein rather than via the direct action of an increase in circulating GLP-1 concentrations.

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