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# Evaluation of the association between plasma glucose-dependent insulinotropic polypeptide, respiratory quotient, and intramuscular fat deposition in feedlot cattle fed different levels of dry matter intake

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## ABSTRACT

The objectives of this trial were to evaluate the association between different levels of dry matter intake (DMI) on gas exchange, plasma glucose-dependent insulinotropic polypeptide (GIP) concentration, and intramuscular (IM) fat deposition. We used 60 individually fed backgrounded Angus × SimAngus-crossbred steers ($n$ = 30) in a randomized complete block design. Steers (paired by body weight [BW] and gain to feed ratio [G:F]) were randomly allocated to one of the following treatments: ad libitum intake (AI) or restricted intake (RI; the same diet fed at 85% of the AI) of a finishing diet. The diet contained 61% cracked corn, 9% corn silage, 15% distillers' dried grains with solubles, 5% soyhulls, and 10% of a protein-mineral-vitamin premix. Measurements of $CO_2$ emission and consumption of $O_2$, and respiratory quotient (RQ) were taken using the GreenFeed system ($n$ = 15/treatment). Plasma and gas samples were collected 10 d before slaughter, 1 h before and 2 h after feeding. Plasma glucose, non-esterified fatty acids, GIP, and insulin concentration and gasses ($O_2$, $CO_2$, and RQ) were analyzed using the MIXED procedure of SAS evaluating the fixed effect of treatment, time (repeated measurement) and their interaction, and the random effect of the block. Final BW and carcass characteristics were analyzed with a similar model, without the time statement and its interaction. Compared with RI, AI steers had greater ($P < 0.01$) DMI and average daily gain (ADG). Steers on AI had greater final BW ($P = 0.02$), tended to have a greater ribeye area ($P = 0.09$), and had lower plasma GIP concentration ($P = 0.04$). There was no treatment effect ($P \geq 0.11$) on G:F, subcutaneous backfat (BF), and IM fat, $O_2$ consumption, $CO_2$ emission, and RQ. Plasma glucose concentration of AI steers was greater before and after feeding than RI ($P < 0.05$). In conclusion, feeding steers ad libitum increased DMI, ADG, and plasma glucose and GIP concentration but does not affect G:F, BF, IM fat, $CO_2$ emission, and $O_2$ consumption. Plasma GIP concentration and RQ are not associated with IM fat deposition.

**Key words:** beef cattle, energy intake, glucose-dependent insulinotropic polypeptide, intramuscular fat deposition, respiratory quotient, restricted intake

## INTRODUCTION

Intramuscular (IM) fat deposition is a desirable trait used to improve meat quality (Nunes et al., 2015; Park et al., 2018; Khan et al., 2019). Increasing IM fat deposition is favorable because it is associated with organoleptic characteristics among which is tenderness and important for optimizing the profitability of the beef cattle industry (Nunes et al., 2015; Grigoletto et al., 2020). To meet the current market demands to improve meat quality, strategies including manipulating nutrition have been implemented in beef cattle, which also has a role in regulating growth performance (Li et al., 2014; Park et al., 2018). Deposition of IM fat has been associated with changes in plasma glucose-dependent insulinotropic polypeptide (GIP) concentration (Freitas et al., 2020).

GIP is a 42 amino acid peptide hormone synthesized in, and released from, K cells (Song et al., 2007; Weaver et al., 2008; Fujii et al., 2014). These K cells are located in the proximal duodenum and jejunum of the gastrointestinal tract (Song et al., 2007; Weaver et al., 2008). Also, GIP is released postprandially; thus, it is released upon nutrient digestion (Song et al., 2007; Fujii et al., 2014). In nonruminants, GIP is secreted when glucose or fat is ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). In ruminants, dietary fatty acids have been suggested to be a potent and a more important nutrient in stimulating GIP compared to dietary starch (Martin and Faulkner, 1993; Martin et al., 1993a; Martin and Faulkner, 1994). Lactating dairy cows were postruminally infused with either corn starch, casein, or soybean oil (Relling and Reynolds, 2008). On day one of infusion, plasma GIP concentration increased when vegetable oil or casein were infused and tended to increase when starch was infused compared with the control (postruminally infused with water). After 7 d of infusion, starch and casein increased plasma GIP concentration. However, cows infused with oil were not different from the control group. Oil infusion decreased dry matter intake (DMI), but did not change metabolizable energy intake compared to the control cows (Relling and Reynolds, 2008). This may suggest that stimulation of plasma GIP concentration was associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control (Relling and Reynolds, 2008). In growing lambs,

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supplementation with lipids in the diet increased plasma GIP concentration in the first week of the experiment, but decreased after 1 month in the animals fed the diet, which might indicate also an adaptation to the fat content in the diet (Relling et al., 2010). Although dietary fatty acid seems to be a potent stimulator of plasma GIP concentration, energy content in the diet and other underlying mechanisms can be an important factors in stimulating GIP.

The main responses of GIP on tissues are stimulating insulin secretion and fat accumulation (Dupre et al., 1973; Martin and Faulkner, 1993; Miyawaki et al., 2002; Yamane et al., 2016). Freitas et al. (2020) reported a positive linear association between plasma GIP concentration and IM fat deposition in feedlot cattle. They did not conclude that GIP increases marbling accretion; however, Martin et al. (1993b) showed that GIP decreased lipolysis in the subcutaneous in sheep. It has been shown that plasma GIP concentration is negatively associated with dairy cows' respiratory quotient (RQ) but positively associated with milk energy output (Relling et al., 2014). Therefore, it is possible that GIP might regulate energy metabolism and partitioning in cattle. Despite the possible importance of GIP in regulating fat deposition, research conducted on GIP and its function in lipid metabolism on ruminants, especially feedlot cattle, is limited.

Based on the literature discussed previously, we hypothesized that animals fed at ad libitum intake (AI), compared to restricted intake (RI), would have greater IM fat deposition; this increase in IM fat deposition is associated with plasma GIP concentration. Animals were given AI have greater plasma GIP concentration and a greater RQ, which means they are using less fat as an energy substrate and have a positive effect on lipogenesis. Therefore, the objective of this experiment was to evaluate the effect of different DMI on plasma GIP concentration and its association with IM fat deposition in feedlot cattle. A second objective was to determine the effect of different levels of DMI on $O_2$ consumption, $CO_2$ emission, RQ, growth, plasma GIP concentration, and carcass characteristics in feedlot cattle.

## MATERIALS AND METHODS

Animal procedures and husbandry practices were approved by the Institutional Animal Care and Use Committee (# 2019A00000112) of The Ohio State University and followed the guidelines recommended in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

### Animals, Experimental Design, and Treatments

The experiment used 60 individually (initial body weight [BW] 399 ± 11.7 kg) fed backgrounded Angus × SimAngus-crossbred steers ($n$ = 30 per treatment) in a randomized complete block design. Steers were weaned at 7 (± 0.35) months of age, preconditioned for 45 days, and moved to the feedlot facility. Animals were housed in individual covered pens (2.6 m × 1.5 m), which consisted of concrete slatted floors, with a 1.5 m long concrete feed bunk, and supplied ad libitum access to clean fresh water. From the arrival to the facility until the experiment started (5 months), the steers were fed a common diet containing 65% of corn silage, 10% of whole shelled corn, 15% of distillers' dried grains with solubles (DDGS), and 10% of a mineral-vitamin premixed. The transition to the experimental (finishing) diet was a step-up increase in corn grain while the corn silage was decreased. The transition to the experimental diet lasted 3 wk.

Seven days before the experiment started, the steers were blocked by BW and gain-to-feed ratio (G:F) during the backgrounding stage. Steers within each block were randomly allocated to ad libitum feed intake (AI) or restricted feed intake (RI). RI steers had the same diet offered at 85% of the AI. The diet contained 61% cracked corn, 9% corn silage, 15% DDGS, 5% soybean hulls, and 10% of a protein-mineral-vitamin premix (Table 1), and it was formulated to meet maintenance and growing requirements of finishing cattle with a grow rate of 1.5 kg/d on the AI steers (NASEM, 2016; Freitas et al., 2020). The level of restriction was chosen to see difference in growth but on animals on a fattening stage. The animals were fed at 0900 h.

### Sampling and Analysis

Feed samples (individual feed ingredients) were collected weekly for feed analysis and DMI was recorded daily. Equal portions of each ingredient were composited and shipped for nutrient composition analysis (Rock River Laboratory Inc., Agricultural Analysis; Wooster, OH). Composite samples were dried and ground through a Wiley mill (1 mm screen, Arthur H. Thomas, Philadelphia, PA). Ingredients were analyzed for dry matter by oven-drying (24 h at 105 °C), neutral detergent fiber (NDF), and acid detergent fiber (Ankom Technology method 5 and 6, respectively; Ankom 200 Fiber Analyzer, Ankom Technology), crude protein (Leco TruMac, LECO Corporation, St. Joseph, MI), ether extract (Ankom method 2; Ankom Technology), and ash (600 °C for 2 h; Thermolyte muffle oven Model F30420C; Thermo Scientific, Waltham, MA). BW was measured on day 0 (day that the animals were fed the finishing diet and the treatments were applied), 29, 49, 75, and before slaughter (average between the day before and the day that the steers were sent to slaughter). These measurements were taken to determine daily DMI, BW,

**Table 1.** Dietary and chemical composition (% DM basis) of a common diet ad libitum or restricted (85% of the ad libitum intake) to finishing steers

| Item | Amount |
|---|---|
| Ingredient, % | |
| Cracked corn | 67.46 |
| DDGS | 15 |
| Corn silage | 9 |
| Soy hulls | 5 |
| Urea | 0.37 |
| Limestone | 1.66 |
| Minerals/vitamins[1] | 1.51 |
| Composition[2] | |
| CP, % | 11.75 |
| NDF, % | 17.58 |
| EE, % | 3.62 |
| Ash | 5.57 |

[1]Mineral and vitamin mix, contained 4.615% of Sodium Chloride, 0.068% of Vitamin A, 30,000 IU/g, 0.068% of Vitamin D, 3,000 IU/g, 0.205% of Vitamin E, 44 IU/g, 6.461% of Ca Sulfate, 0.351% of Selenium, 0.203%, Rumensin 90 (Elanco Animal Health, Greenfield, IN), 2.769% of Potassium Chloride, 0.06% of Copper Sulfate, 0.185% of Zinc Sulfate, 0.111% of Manganese Sulfate, and 0.001% of Cobalt Carbonate.
[2]CP, crude protein; NDF, neutral detergent fiber; EE, ether extract.

average daily gain (ADG), and G:F. Measurements of $CO_2$ and $CH_4$ emissions, $O_2$ consumption, RQ, and plasma samples were collected 10 d before slaughter, 1 h before, and 2 h after feeding. Gases ($n$ = 15/treatment) were measured using the GreenFeed system (C-Lock Inc., Rapid City, SD) as described previously (Hristov et al., 2015; Gunter et al., 2018). On the days that the gas samples were taken, the DMI at 2 h after feeding was collected. The reasoning for this sampling time was based on previous research (Relling and Reynolds, 2008; Ortiz-Fraguada, 2021) where plasma concentration of hormones and gas production was impacted by the diet relative to feeding time. For this reason, the amount of feed that remained in the bunk at 2 h after feeding was collected, weighed, and refed. The reason for these measurements was to evaluate the amount of feed intake at the time of plasma and gas samplings.

During d 7 to 28 of the project, the steers were adapted to the use of the gas sensor equipment (GreenFeed System). Twice a week for 3 wk, each steer was walked to the chute where the GreenFeed system was located. On d 1 of the adaptation, the animals were taken to the chute, kept there for 1–2 min, and walked back to their pens. On d 2 and 3 of the adaptation, the animals were walked to the chute and a bucket was introduced with 50 g of cracked corn. Then, steers remained in the chute for 3 min. On d 4, 5, and 6 of the adaptation, the concentrate was introduced in the GreenFeed system, and the system was moved in front of them. This adaptation was used also to select animals that had a calm behavior during the exposure of the gas sensor equipment. The steers used for gas collection were randomly selected from the ones that both steers for each pair block showed a calm behavior with the used of GreenFeed system. The ratio $CO_2/O_2$ was used to determine the RQ, and as a marker of substrate used to supply energy.

Blood samples were taken after the gas collection times (1 h before, and 2 h after feeding) via the jugular vein to measure glucose, non-esterified fatty acids (NEFA), GIP, and insulin. Blood samples were immediately transferred to tubes containing solutions of disodium ethylenediaminetetraacetic acid and benzamidine HCl (1.6 and 4.7 mg/mL of blood, respectively) and placed on ice. After centrifugation for 30 min (1,800 × $g$ and 4 °C), blood was aliquoted into individual polypropylene tubes and stored at −80 °C until analyzed. After the BW on day 75, animals were visually appraised and set a target finishing day. During d 90 to 115, animals (15 steers/treatment) were harvested commercially depending on the amount of backfat (BF; visual appraisal) of the steer in the AI of each pair block. Steers in the RI treatment were sent to slaughter at the same time when the AI steers reached visually the amount of BF targeted. Hot carcass weight (HCW) was recorded on day of slaughter. A sample from the 12th to 13th rib of the *Longissimus dorsi* muscle (LM) was collected at harvest for later analysis of LM area, BF, and IM fat concentration. IM fat was measured using ether extract (AOCS, 2005). The LM area was determined for the LM at the 12th and 13th rib interface with tracing techniques. A single individual outlined the ribeye area with a fine point marker using transparent vellum paper. The tracings were scanned using an Epson Perfection V500 photo scanner (Epson America, Inc., Long Beach, CA). A 5.08 cm × 5.08 cm square outline was included in each scan for calibration purposes. The tracings were quantified in Adobe Photoshop (Adobe Photoshop 2020, Adobe Creative Cloud and Acrobat, San Jose, CA).

Plasma insulin concentration was measured using radioimmunoassay (RIA) as described by Miqueo et al. (2019). Plasma GIP concentration was measured using a modified RIA based on the Phoenix pharmaceutical assay kit (Phoenix pharmaceutical RK-027-02). The modification of the assay was half of the volumes used. The antibody of this assay was validated previously for cattle (Relling et al., 2014). 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 intra assay variation was below 8% for all the assays.

### Statistical Analysis

The experiment was analyzed as a complete randomized block design. Plasma glucose, NEFA, GIP, insulin concentration, and gases ($O_2$, $CO_2$, and RQ) were analyzed using the MIXED procedure of SAS (9.4) with repeated measurements. The model evaluated the fixed effect of treatment, time (repeated measurement), their interaction, and the random effect of the block. The animal was the subject, and the most appropriate covariance structure was chosen as having the lowest Akaike Information Criterion. The first-order autoregressive covariance structure was used for the analysis of RQ and plasma glucose and GIP concentrations. The unstructured covariance structure was used for analysis of plasma insulin and NEFA concentrations. The compound symmetry covariance structure was used for the analysis of $O_2$ consumption and $CO_2$ emission. Because of the lack of treatment by time interaction ($P \geq 0.15$) on DMI, ADG, and G:F, only the main effect of treatment is presented in the tables. Final BW, DMI at 2 h after feeding, and carcass characteristics were analyzed with a similar model without the time statement and its interaction. Days on feed were used as a covariate for final BW. A significance was declared at $P \leq 0.05$ and tendencies were considered at $0.05 < P \leq 0.10$.

## RESULTS AND DISCUSSION

### Growth Performance

Steers on AI have greater final BW ($P$ = 0.02; Table 2). This greater final BW in AI steers may be due to the increase in DMI during the finishing period and it is similar to what was observed previously with growing cattle fed a grass silage diet

**Table 2.** Mean ± SEM for body weight (BW), dry matter intake (DMI), average daily gain (ADG), gain to feed ratio of backgrounded beef cattle fed a finishing diet ad libitum and restricted intake, similar diet but at 85% of the ad libitum intake, during the finishing phase in a feedlot[1]

| Items | Ad libitum | Restricted | SEM | *P*-value |
|---|---|---|---|---|
| Animals | 30 | 30 | — | — |
| Initial BW, kg | 398.7 | 399.4 | 11.88 | 0.93 |
| Final BW, kg | 546.1 | 522.4 | 6.96 | 0.02 |
| Daily DMI, kg/d | 7.66 | 6.39 | 0.297 | < 0.01 |
| 2 h, DMI, kg[2] | 5.13 | 6.64 | 0.321 | < 0.01 |
| ADG, kg | 1.32 | 1.13 | 0.056 | 0.01 |
| Gain:feed ratio | 0.178 | 0.180 | 0.0135 | 0.77 |

[1]Backgrounded cattle. Diets contained 9% corn silage, 61% cracked corn, 15% DDGS, 5% soyhulls, and 10 % of a protein-mineral-vitamin premix on a DM basis.
[2]2 h, DMI is the amount of DMI at the 2 h after the feed was offered ($n$ = 15) during the days that gas was sampled. Gasses were sampled 10 d before slaughter.

that had increasing final BW with increasing intake level (ad libitum vs. 80% and 65% restriction; Ouellet et al., 2001). Similarly, growing cattle fed ad libitum grass silage had greater final BW than restricted-fed calves (80%) (Prezotto et al., 2017).

Compared to RI, AI steers had greater ($P < 0.01$; Table 2) daily DMI and ADG. By design, restricted animals consumed less feed than ad libitum fed animals. However, the DMI after 2 h of feed offered, on the days that gas exchange and blood samples were taken, was greater ($P < 0.01$; Table 2) for the RI steers compared with the AI steers. Similar differences in ADG due to diet restrictions have been reported previously (Hicks et al., 1990; Holt et al., 2000; Ouellet et al., 2001; Prezotto et al., 2017). The differences in DMI 2 h after feeding, where RI steers eat more than the ad libitum feed steers, might be due to changes in eating behavior. In previous studies, this difference was observed, however, not reported (Relling et al., 2010; Dr. S. C. Loerch, personal communication). A possible cause for the increase in DMI in those 2 h and change in eating behavior might be due to the metabolic/chemostatic regulation of DMI (Relling et al., 2010; Sartin et al., 2011) as observed for the lesser pre-feeding glucose concentration in the RI compared with the AI feed steers.

There was no effect of treatment ($P = 0.77$) on G:F. The lack of difference in efficiency between the treatments is similar to other experiments (Hicks et al., 1990; Hayden et al., 1993; Holt et al., 2000). Mcgregor et al. (2012) reported no differences in G:F in steers fed ad libitum a high concentrate diet compared with restricted fed steers (77% of the ad libitum) for 84 d. Feed-to-gain ratio (F:G) was less for steers fed a 90% haylage diet compared to steers fed a high concentrate diet (Mcgregor et al., 2012). The authors (McGregor et al., 2012) discussed that the improvement in efficiency in limit-fed and then fed ad libitum steers was because they reduce maintenance costs and improve diet digestibility compared to ad libitum fed steers. However, greater efficiency (lesser F:G) was seen in crossbred steers fed 70% to 80% of ad libitum DMI that transitioned to ad libitum DMI for an additional 20 days (Holt et al., 2000). Holt et al. (2000) suggested that the contrasting results in efficiency were because of differences in diet digestibility and that restricted-fed cattle have previously shown a greater efficiency of metabolizable energy use for body energy gain. Murphy et al. (1994) reported that mild restrictions in DMI resulted in improvements in digestibility in steers fed a corn silage-based diet. The observed results in G:F differ from previous research limiting feed intake of a forage-based diet. There was an improvement in G:F when restricting intake was reported in growing calves fed a forage-based diet for 84 d ad libitum (Prezotto et al., 2017). Prezotto et al. (2017) reported that ad libitum DMI steers had greater G:F than those limit-fed at 80% of the ad libitum DMI. Prezotto et al. (2017) discussed that the results of limit-feeding may differ depending on the level of restriction, type of diet (forage compared with concentrate), and energy density of the diet. Those factors are possible explanations for when Ouellet et al. (2001) noted that F:G decreased linearly with increasing levels of DMI in growing cattle fed a grass silage diet, which meant ad libitum animals were more efficient; however, animals had a different diet and level of restriction (80% and 65%) compared to the present experiment.

## Plasma Hormone and Metabolite Concentration

No time by treatment interactions ($P \geq 0.13$) were observed for plasma hormones and metabolites concentrations. Plasma glucose concentration of AI steers was greater compared with RI steers ($P = 0.02$; Table 3). Pre-feeding plasma glucose concentration was greater (Time effect $P = 0.01$) than post-feeding plasma glucose concentration. Pre-feeding plasma insulin concentration was lesser (time effect $P < 0.01$) than post-feeding plasma insulin concentration. There was no effect of treatment ($P \geq 0.45$) on plasma insulin and NEFA concentration. However, steers tended to have a time effect on plasma NEFA concentration ($P = 0.06$); where pre-feeding plasma glucose concentration was greater than post-feeding plasma glucose concentration. There was an effect of time and treatment on GIP ($P \leq 0.04$). Plasma GIP concentration was greater after eating and greater in AI animals.

The plasma glucose concentration of AI steers is similar to the one reported by Becú-Villalobos et al. (2007) in Angus and Angus–Hereford feedlot steers fed a high concentrate diet (40.6% corn grain) for 84 d. The time effect on plasma

**Table 3.** Mean ± SEM plasma glucose, insulin, glucose-dependent insulinotropic polypeptide (GIP), and non-esterified fatty acids (NEFA) concentration, $O_2$ consumption, $CO_2$ emission, $CH_4$ emission, and RQ from beef cattle fed ad libitum or restricted diets during the finishing phase in feedlot[1]

| Items[2] | Ad libitum | | Restricted | | SEM | *P*-values | | |
|---|---|---|---|---|---|---|---|---|
| | Pre[3] | Post[4] | Pre[3] | Post[4] | | Treatment | Time | *T* × *I*[5] |
| Animals, *n* | 15 | 15 | 15 | 15 | — | — | — | — |
| Glucose, mM | 4.9 | 4.4 | 4.3 | 3.9 | 0.26 | 0.02 | 0.01 | 0.54 |
| Insulin, pmol/mL | 79 | 93 | 65 | 97 | 11.6 | 0.66 | < 0.01 | 0.13 |
| GIP, pmol/mL | 65 | 76 | 46 | 52 | 7.2 | 0.01 | 0.04 | 0.47 |
| NEFA, µM | 136.7 | 132.1 | 133.6 | 123.4 | 6.61 | 0.45 | 0.06 | 0.46 |
| $CO_2$, g/d | 13,178 | 11,954 | 11,858 | 11,502 | 498.47 | 0.11 | 0.04 | 0.24 |
| $O_2$, g/d | 9,072.90 | 8,742.00 | 8,223.70 | 8,548.13 | 320.10 | 0.16 | 0.99 | 0.12 |
| $CH_4$, g/d | 15.67 | 38.93 | 14.30 | 39.15 | 4.62 | 0.88 | < 0.01 | 0.79 |
| RQ | 0.689 | 0.738 | 0.696 | 0.745 | 0.0189 | 0.66 | < 0.01 | 0.98 |

[1]Samples were collected 10 d before slaughter.
[2]GIP, glucose-dependent insulinotropic polypeptide; NEFA, non-esterified fatty acids; RQ, respiratory quotient.
[3]Pre = 1 h before feeding.
[4]Post = 2 h after feeding time.
[5]*T* × *I* = interaction of treatment and time main effects.

glucose and insulin concentration is consistent with established data (Brockman and Laarveld, 1986; Becú-Villalobos et al., 2007). Blood glucose regulation is integrated with the regulation of other metabolic processes through common hormones such as insulin (Brockman and Laarveld, 1986). Plasma insulin concentration was greater and plasma glucose concentration was lesser than 2 h after feeding in both treatments and may be because plasma insulin concentration is positively correlated with feed intake and facilitates glucose and amino acid uptake by peripheral tissues (Brockman and Laarveld, 1986). Plasma glucose concentration was lesser 2 h after feeding, perhaps because insulin increases the uptake of glucose into peripheral tissues such as muscle and fat (Brockman, 1978; De Koster and Opsomer, 2013). Ouellet et al. (2001) reported using a grass silage diet that preprandial plasma glucose concentration was lesser in ad libitum-fed cattle compared to limit fed cattle (80% and 65% restrictions) after 79 d; also, plasma glucose concentration decreased with feeding and was greater in restricted animals. In the present experiment, plasma glucose concentration did decrease with feeding but was greater in ad libitum animals. A possible explanation for this difference may be the different types of diets. Ouellet et al. (2001) used a forage-based diet; however, the current experiment used a high grain diet.

Ouellet et al. (2001) reported that plasma insulin concentration before feeding was greater in ad libitum animals compared to feed restricted steers fed a grass silage (timothy or bromegrass) diet after 78 d; which contradicts the results of the present experiment from which there was no treatment or a time by treatment interaction effect. However, Ouellet et al. (2001) also reported that, on d 79, plasma insulin concentration 2 h after feeding was greater in ad libitum-fed animals compared to restricted-fed animals which contrasts with this experiment. Therefore, it is possible that the changes in insulin concentration do not depend just on the amount of feed consumed by the cattle, but also by the type of feed, where a high grain diet with restricted DMI, such as in the current experiment, may be able to stimulate insulin secretion, similar to animals fed ad libitum.

Pre-feeding greater plasma NEFA concentration could be explained based on the need for energy; NEFA are released into circulation from the adipose tissue to meet the metabolic needs of the animals (Bowden, 1971; De Koster and Opsomer, 2013). Plasma NEFA concentration is also associated with lesser insulin concentration (Bowden, 1971; De Koster and Opsomer, 2013). This is similar to previous research reporting that in ruminants plasma NEFA concentration is related primarily to the time of feeding (Bowden, 1971). These observed changes in the concentration of plasma NEFA could also be related to the increase in plasma insulin concentration after feeding; because it has been reported that insulin could inhibit the release of NEFA in ruminants (Bowden, 1971). Also, in growing lambs, a negative association was reported between the plasma concentration of GIP and NEFA (Relling et al., 2010). The authors discussed that the negative association between plasma GIP and NEFA concentration could possibly be due to a role reported in GIP in decreasing lipolysis in the subcutaneous adipose tissue (Martin et al., 1993b). Greater NEFA concentration indicate lipid mobilization and fatty acid oxidation (Wathes et al., 2009). The observed results of plasma NEFA concentration differ from growing cattle fed a grass silage diet, in which there was an effect of time and intake on plasma NEFA concentration (Ouellet et al., 2001). Restricted-fed cattle (80% and 65%) had greater plasma NEFA concentration before and after feeding compared to the ad libitum fed cattle (Ouellet et al., 2001). Also, in that experiment, plasma NEFA concentration on all treatments decreased after feeding (Ouellet et al., 2001). The authors discussed that animals with greater intakes had lesser plasma NEFA concentration because they received more silage, and therefore, a greater nutrient supply (Ouellet et al., 2001). A difference from Ouellet et al. (2001) and the current experiment is that the steers in the current experiment were fed a high-grain diet; therefore, despite that, a group was fed restricted, it is possible that it had less fatty acid mobilization than the steers feed restricted in a high forage diet.

The results of the current experiment support our hypothesis that steers fed AI would have greater plasma GIP concentration than the RI steers. Plasma GIP concentration was greater after feeding time, which is in agreement with established data that GIP is released upon nutrient digestion (Relling and Reynolds, 2008; Relling et al., 2010). In steers fed at AI, plasma GIP concentration was greater compared to RI animals, despite that DMI of RI steers was greater than AI steers 2 h after feeding. Similar results on total daily energy intake were reported in lactating dairy cows that were post-ruminally infused with either corn starch, casein, or soybean oil (Relling and Reynolds, 2008). On d 7, starch and casein increased plasma GIP concentration. This suggests that stimulation of plasma GIP was associated with the increase in total metabolizable energy supply, which was greater in casein and starch infusions compared to the control (Relling and Reynolds, 2008). The observed results are similar to those reported in non-ruminants because GIP was secreted when glucose was ingested (Kim et al., 2007; Weaver et al., 2008; Tharp et al., 2020). Previously, lipids, but not energy, were thought to be an important nutrient in stimulating GIP in ruminants (Martin and Faulkner, 1993; Martin et al., 1993a; Martin and Faulkner, 1994). Results in the present experiment could indicate that energy content in the diet is still a factor in stimulating plasma GIP concentration in ruminants since there was no fat supplemented in the diet and the animals were fed similar diets. The possible differences in plasma GIP concentration after feeding, despite the RI, had a greater DMI, could be because the baseline (pre-feeding) in the RI steers was lower, and despite the extra intake of energy was not enough to produce a change to reach similar concentrations than the AI. The 2 h after feeding sampling time may not be enough for the greater intake of nutrients to pass to the small intestine and stimulate GIP secretion in the RI steers (Aikman et al., 2008).

## Gas Exchange

We did not observe a treatment or treatment × time interaction effect ($P \geq 0.11$; Table 3) on $O_2$ consumption, RQ, $CO_2$, and $CH_4$ emission. There was a time effect for $CO_2$ ($P < 0.01$), where $CO_2$ emission was greater pre-feeding than post-feeding. After feed ingestion, the oxidation of glucose through cellular respiration, the animal should produce more $CO_2$, which is what was expected to occur 2 h after the feed was offered in the present experiment. However, the decrease of postprandial $CO_2$ emissions cannot be explained or supported with existing literature.

Main effect of time ($P < 0.01$) was observed on $CH_4$ emission (Table 3). Post-feeding $CH_4$ emission was greater than pre-feeding for both treatments. A possible explanation for the time effect on $CH_4$ emission is because it is a by-product of microbial fermentation of carbohydrates (Hristov et al., 2013).

We had hypothesized that animals fed AI would have greater plasma GIP concentration and greater RQ. As described by Patel et al. (2022), RQ is the ratio between $CO_2$ released and $O_2$ consumption, yielding values ranging from 1, when only carbohydrates are oxidized, to 0.7, when only lipids are oxidized. Therefore, an increase in RQ for the current experiment indicates that the steers will be using less fat as energy substrate, and this will promote an increase in lipogenesis. The main responses of GIP in non-ruminants are stimulating insulin secretion and to increase fat accumulation (Dupre et al., 1973; Martin and Faulkner, 1993; Miyawaki et al., 2002; Yamane et al., 2016). The steers in both groups had lesser RQ before feeding at approximately 0.7 which could indicate that fat was used as an energy substrate (Rogobete et al., 2019). Nonetheless, RQ results before and after feeding were approximately 0.7 which mean they were still using mainly fat as a substrate. Something worth to comment is that the RQ values were obtained as two samplings (pre- and post-prandial) and do not reflect the whole day RQ but the values relative to the sampling for these two specific treatments.

### Carcass Characteristics

We had hypothesized that feeding AI to beef steers would increase IM fat deposition; however, there was no treatment effect ($P \geq 0.11$; Table 4) on HCW, BF, and IM fat. Steers on AI tended to have greater LM area ($P = 0.09$) compared with steers on RI. A lack of difference in LM area, IM fat, and HCW was observed when comparing different DMI in Continental and British steers fed a high moisture corn diet ad libitum or limit-fed (77%) for 84 d (Mcgregor et al., 2012). Similarly, steers fed a high-wheat diet ad libitum or restricted for 149 d had no differences in HCW, LM area, or BF (Hicks et al., 1990). Also, Hereford steers fed a high-corn diet ad libitum for 138 d tended to have greater HCW, but no differences in LM area or BF compared with restricted-fed animals (restricted during 56 d followed by a period of ad libitum feeding for 82 d; Hicks et al., 1990). Results in the present experiment differ from growing cattle that had greater carcass weight with increasing feeding level (Ouellet et al., 2001). However, the experiment was during the growing period with a silage-based feed which contrasts from the present experiment that fed steers a high-concentrate diet during the finishing period (Ouellet et al., 2001). Plasma GIP concentration has been associated with IM fat deposition (Freitas et al., 2020); however, we did not observe an association between these variables. As we are aware, the present experiment and the one from Freitas et al. (2020) are the only experiments that associate plasma GIP concentration with IM fat deposition. Despite both experiments are in finishing Angus crossbred cattle fed high corn diet, the current experiment has less protein concentration and greater NDF concentration than the diet of the experiment from Freitas et al. (2020). Therefore, there might be factors associated to the diet, time of sampling that might influence GIP secretion and function.

**Table 4.** Mean ± SEM hot carcass weight (HCW), backfat, intramuscular (IM) fat, and *Longissimus dorsi* muscle (LM) area for ad libitum and restricted fed steers given a concentrate diet[1]

| Items[2] | Ad libitum | Restricted | SEM | *P*-value |
|---|---|---|---|---|
| Animals | 15 | 15 | — | — |
| HCW[3], kg | 344 | 335 | 6.5 | 0.15 |
| Back fat, cm | 1.81 | 1.56 | 0.167 | 0.11 |
| IM fat[2], % | 6.28 | 5.96 | 0.488 | 0.53 |
| LM[4] area, cm$^2$ | 79.1 | 74.1 | 2.66 | 0.09 |

[1]Backgrounded cattle. Diets contained 9 % corn silage, 61% cracked corn, 15% DDGS, 5% soyhulls, and 10 % of a protein-mineral-vitamin premix on a DM basis.
[2]IM fat, ether-extractable intramuscular fat; LM, *Longissimus dorsi* muscle; HCW, hot carcass weight.

## Conclusions

In conclusion, feeding steers at ad libitum increased ADG and plasma glucose and GIP concentration, but did not affect G:F, BF, IM fat, $CO_2$ emission, RQ, and consumption of $O_2$. Despite previous experiments mention that GIP concentration is only increased by the addition of lipids, results in the present experiment indicate that plasma GIP concentrations in ruminants could also be stimulated by metabolizable energy content in the diet. Although there was a significant effect of treatment on plasma GIP concentration, there were no differences in RQ or IM fat deposition. Therefore, the association of GIP and IM fat deposition cannot be confirmed based on the data of the current experiment.

## Conflict of Interest Statement

The authors declare that there is no known conflict of interest.

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