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Relationship of circulating ghrelin and leptin concentrations in beef cattle exhibiting differences in composition of gain[1]

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SUMMARY

Beef steers (n = 72) of similar age, weight (651 ± 3.1 lb), and genetic background (Angus crossbred) were used to determine the effects of growing period diet on the relationship of plasma ghrelin and leptin concentrations with growth performance and carcass composition. At trial initiation (d 0), 8 steers were harvested for initial carcass composition. The remaining 64 steers were allotted, by weight, to pen and treatment was assigned randomly. Treatments were 1) a high-forage diet fed during the growing period (0-116 d) followed by a high-concentrate diet during the finishing period (117-209 d; GRW-FNSH) or 2) a high-concentrate diet fed for the duration of the trial (0-209 d; FNSH-FNSH). Steers were allowed ad libitum consumption regardless of dietary treatment. Eight steers per treatment (1 pen) were harvested on d 88, 116, 165, and 209. At each harvest date, 9-10-11th rib sections were dissected for chemical composition, and carcass characteristics were recorded. Replicate blood samples were collected from every steer prior to each harvest, and assayed for ghrelin, leptin, GH, insulin, and NEFA concentrations. Hormone, growth performance, and carcass composition were analyzed statistically using the GLM procedure of SAS to evaluate diet, harvest date, and their interaction. Linear, quadratic, and cubic contrasts were performed. Percent carcass protein decreased linearly ($P < 0.001$) and percent carcass fat increased linearly ($P < 0.001$) in both treatments. At each harvest day, FNSH-FNSH steers had greater carcass fat ($P < 0.01$) compared with GRW-FNSH steers. Plasma ghrelin concentrations for FNSH-FNSH increased quadraticly ($P < 0.001$) over time, whereas plasma ghrelin concentrations were not different over time for GRW-FNSH. Plasma leptin concentrations for FNSH-FNSH increased ($P < 0.001$) from d 0 to 88 and then plateaued, whereas plasma leptin concentrations increased linearly ($P < 0.001$) for the GRW-FNSH. Plasma ghrelin and leptin concentrations fluctuated relative to nutritional status, and plasma ghrelin concentrations were highest in excessively fat cattle. The role of ghrelin during fat accumulation warrants further investigation.

INTRODUCTION

The balance of energy intake and expenditure influences composition of gain thus resulting in more or less carcass fat relative to lean tissue. Premiums and discounts for beef carcass are established on the basis of intramuscular fat (quality grade) and the ratio of fat to lean tissue (yield grade). Peptide

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hormones leptin and ghrelin recently have been reported to be involved in regulation of feed intake and (or) body composition in cattle (Keisler et al., 1999; Wertz-Lutz et al., 2006). As fat cells increase in mass, peripheral concentrations of leptin also increase (Delavaud et al., 2002). Ghrelin is a peptide hormone synthesized by abomasal and ruminal tissues of cattle (Hayashida et al., 2001; Gentry et al., 2003) and concentration increases with feed restriction (Wertz-Lutz et al., 2006 and 2008). In addition, ghrelin influences energy metabolism (Tschöp et al., 2000) and could directly influence the deposition of fat (Patel et al., 2006). While leptin has been reported to fluctuate relative to body composition in cattle, fluctuations of ghrelin relative to body composition remains unknown. More specifically, a relationship between circulating leptin and ghrelin concentrations in beef cattle with differential composition of gain has yet to be established. We hypothesize that when compared with steers grown on a high-energy diet throughout the growing and finishing period, plasma leptin:ghrelin will be decreased for steers on a low-energy diet during the growing period. Steers consuming the low-energy diet will have less body fat, thus decreasing leptin concentration. However, ghrelin concentration will be elevated as nutrient intake will be less than the cattle's potential for growth. Therefore, objectives for this experiment were to 1) Establish a relationship of plasma leptin and ghrelin concentrations in cattle exhibiting differential composition of gain and 2) Evaluate the relationship of plasma leptin and ghrelin concentrations with differences in carcass composition.

MATERIALS AND METHODS

Animals

Experimental procedures were approved by the South Dakota State University (SDSU) Laboratory Animal Resource Committee on Animal Care. Seventy-two beef steers of similar age (8 mo), weight (651 ± 3.1 lb), and genetic background (Angus crossbred) were used in this experiment. Steers used in the experiment were not implanted during the growing or finishing period. At trial initiation (d 0), blood samples were collected to establish baseline plasma hormone and metabolite concentrations, and 8 steers were harvested for initial carcass composition analyses. The remaining 64 steers were allotted, by weight to eight pens, and a growing period treatment was assigned randomly. Live weights were recorded on two consecutive days and averaged to establish initial BW. Intermediate weights were recorded at 28-d intervals to monitor growth.

Dietary Treatments

Ingredient and nutrient composition of diets are presented in Table 1. Dietary treatments were 1) a low-energy diet (GRW1 and GRW2) for the growing period (d 0-116) followed by a high-energy diet during the finishing period (d 117-209; GRW-FNSH) or 2) a high-energy diet (FNSH) for the duration of the experiment (d 0-209; FNSH-FNSH). Steers were fed twice daily (8:00 am and 3:00 pm) and were allowed ad libitum access to feed and water throughout the trial. Steers assigned to the FNSH-FNSH treatment received a diet formulated to target 3.5 lb/d rate of gain (NRC, 2000). At the 83-d intermediate weight, steers assigned to the GRW-FNSH treatment were gaining faster (3.0 lb/d) than NRC equations predicted. As a result, the low-energy diet (GRW1) was adjusted by increasing the grass hay content to 25% and lowering the dry-rolled corn content to 13% (GRW2), thus lowering the energy content from 0.50 to 0.45 Mcal of NEg/lb (DM basis). To achieve the desired target, the GRW2 diet was then fed to the GRW-FNSH steers from d 89 to 116, followed by the FNSH diet from d 117 to 209.

Eight steers from each treatment group (1 pen) were harvested at 4 points in the growth trial. Harvest points were: 1) during the growing period (d 88), 2) the end of the growing period (d 116), 3) the point

at which the FNSH-FNSH steers reached 0.4 in ribfat (d 165), and 4) the point at which the GRW-FNSH reached 0.4 in ribfat (d 209). To monitor targeted ribfat, ultrasound measurements were recorded periodically before harvest points 3 and 4.

Table 1. Dietary ingredient and nutrient content of diets [a]

	Dietary Treatments		
Item	GRW1	GRW2	FNSH
High moisture corn	-	-	26.3
Whole shell corn	-	-	53.7
Dry rolled corn	28.0	13.0	-
Soybean meal	5.0	5.0	5.8
No grain corn silage	50.0	50.0	10.0
Grass hay	10.0	25.0	-
Liquid supplement [b]	-	-	4.3
Supplement [b]	7.0	7.0	-
Calculated nutrient composition			
DM	42.4	42.6	67.8
Ca	0.39	0.47	0.63
P	0.32	0.31	0.34
CP	12.5	12.5	12.5
NE_m, Mcal/lb	0.80	0.75	1.23
NE_g, Mcal/lb	0.50	0.45	0.63

[a] % Dry matter basis.

[b] Provided vitamins and minerals to meet or exceed nutrient requirements (NRC, 2000), FNSH diet contained 29 g/T of Rumensin and GRW diets contained 25 g/T of Rumensin.

Serial Blood Sample Collection

Blood samples were collected before each scheduled harvest point. Wertz-Lutz et al. (2006) demonstrated that plasma ghrelin concentrations fluctuate relative to nutritional status and can be pulsatile during acute feed deprivation. As a result, blood samples were collected beginning 4 h after the steers had been offered feed (12:00 pm), and two samples from each steers were collected 15 min apart. Blood samples were collected into tubes containing K_3-EDTA, placed on ice, and then centrifuged at 4°C for 30 min at 1,100 x *g*. Plasma was separated into 1-mL aliquots for subsequent analysis of leptin according to Delavaud et al., (2000), and ghrelin, GH, insulin (INS), and NEFA concentration according to Wertz-Lutz et al. (2008).

Serial Carcass Data

Steers were transported from the SDSU Beef Nutrition Unit to the SDSU abattoir on the day of harvest. Eight steers per treatment were harvested at designated points throughout the growth trial. Hot carcass weights (HCW) were recorded, and carcasses were allowed to chill for 48 h and then ribbed between the 12^{th} and 13^{th} rib. Ribeye area (REA), ribfat (RF), marbling score (MARB), and KPH fat were recorded, and calculated yield grade (YG), and dressing percentage (DP) determined.

Serial Carcass Composition and Analyses

Following the collection of standard carcass characteristics, 9-10-11 rib sections were removed from the right side of each carcass according to the procedures of Hankins and Howe (1946). Each rib section was weighed, lean and fat tissues were removed from the bone, and each tissue was weighed separately. The lean and fat tissue were homogenized, and the homogenate stored at -20°C for subsequent analyses of DM, ether extract (EE), and crude protein (CP) content for determination of carcass composition (AOAC, 2007). Hankins and Howe (1946) equations for steers were used to estimate carcass composition from chemical composition of the 9-10-11 rib sections. Equations were as follows: percent carcass fat = 3.49 + 0.74(9-10-11 rib soft tissue %EE), percent carcass protein = 6.19 + 0.65(9-10-11 rib soft tissue %CP). A 1-in-thick portion of the *longissimus dorsi* was removed from the 12th rib section of the right side of each carcass. Processing and ether extraction of the *longissimus dorsi* sample was performed to determine intramuscular fat (IMF) content and were the same as those outlined previously with the 9-10-11 rib section.

Statistical Analyses

Seventy-two steers were used at trial initiation. However, during the feeding period one steer from each treatment was removed due to illness unrelated to dietary treatment. Therefore, statistical analyses were performed on data from the remaining 70 steers. Individual steer was used as experimental unit. Because cattle were harvested throughout the experiment, the number of replications per treatment decreased at each serial harvest point. Data were analyzed as a randomized complete design using the GLM procedure of SAS. Differences in treatments means that resulted treatment, harvest date and their interaction were separated using least squares means with the PDIFF option. Mean differences were considered significant at $P < 0.05$. Orthogonal polynomials for unequal spacing of serial harvest points were established using the IML procedure of SAS. These polynomials were used to establish linear, quadratic, and cubic relationships between harvest dates.

RESULTS AND DISCUSSION

Growth Performance of Steers

Steer BW differed as a result of dietary treatment during the growing and finishing period. Body weights were lower ($P < 0.001$) in the GRW-FNSH by the end of the growing period (d 116; 983 vs. 1051 ± 9.3 lb, respectively) compared with the FNSH-FNSH steers. During the finishing period (d 165; 1149 vs. 1191 ± 7.7 lb, respectively) and at the final harvest point (d 209; 1308 vs. 1283 ± 18.3 lb, respectively) BW were similar between treatment groups. Both treatment groups exhibited a linear ($P < 0.001$) increase in BW over time. Steers in the FNSH-FNSH treatment group had greater ADG ($P < 0.05$) during the growing period (Figure 1A). In contrast, the GRW-FNSH steers had an increased ADG ($P < 0.05$) during the finishing period. Whereas, cumulative ADG for both treatment groups was similar for the entire trial (Figure 1B), the point where accelerated growth rate occurred differed by treatment.

Carcass Composition

Differences in rate of gain altered body composition between treatment groups. Percent carcass fat increased linearly ($P < 0.001$) over time for both FNSH-FNSH and GRW-FNSH steers (Figure 2A). At each harvest day, FNSH-FNSH steers had greater carcass fat ($P < 0.01$) compared with GRW-FNSH steers. Percent carcass protein decreased linearly ($P < 0.001$) for both GRW-FNSH and FNSH-FNSH cattle (Figure

2B). Despite a similar rate of decline between treatment groups, the percentage of the carcass composed of protein was greater ($P < 0.01$) in the GRW-FNSH steers. Fox et al. (1972) observed similar results where compensating steer gains were higher in protein and lower in fat content earlier in the realimentation period. These data suggest that increased rates of gain during compensation are composed initially of protein whereas cattle on an uninterrupted plane of nutrition are partitioning energy toward fat. Percent IMF content for the FNSH-FNSH increased quadraticly ($P < 0.01$) compared with a linear ($P < 0.001$) increase for the GRW-FNSH steers (Figure2C). These data suggest a high energy diet earlier in the growing period allowed the FNSH-FNSH steers to reach their genetic potential of marbling. Bruns et al. (2004) also demonstrated marbling develops early and continuously if the cattle are exposed to excess energy availability early in development. Data from the current experiment supports the hypothesis that the potential to deposit IMF can be influenced by diet during the growing period.

Carcass Characteristics

For GRW-FNSH steers, HCW were lower ($P < 0.001$) during the growing period restriction but converged and were not significantly different from FNSH-FNSH steers HCW at final harvest (Table 2 and 3). Ribeye area increased ($P < 0.001$) similarly over time in both treatment groups. Yield grade was consistently higher ($P < 0.001$) for FNSH-FNSH steers compared with GRW-FNSH steers, which supports increased fat relative to lean tissue in the FNSH-FNSH treatment group. An interaction of dietary treatment and serial harvest day resulted for marbling scores ($P < 0.001$). Marbling scores increased at a greater rate for FNSH-FNSH steers compared with GRW-FNSH steers. Ribfat increased ($P < 0.001$) throughout the feeding period in both dietary treatments.

Table 2. Standard carcass characteristics of initial harvest group

Variable		SEM
Carcass characteristics [a]		
No.	8	
DP, %	52.8	0.32
HCW, lb	341	12.8
REA, in^2	7.21	0.19
RF, in	0.08	0.02
MARB [b]	224	28.00
YG	2.09	0.10

[a] DP = dressing percent, REA = ribeye area measurement, RF = ribfat measurement, MARB = marbling score, and YG = calculated yield grade.

[b] Marbling scale; practically devoid = 100, traces = 200, slight = 300, small = 400, modest = 500, moderate = 600, slightly abundant = 700, moderately abundant = 800, abundant = 900.

Fluctuations of Plasma Hormones and Metabolites during the Feeding Period

Plasma NEFA concentrations were higher ($P < 0.05$) in the GRW-FNSH steers during the growing period but were similar during the finishing period compared with the FNSH-FNSH steers (Figure 3A). Plasma INS concentrations were increased ($P < 0.05$) in the FNSH-FNSH steers compared with the GRW-FNSH steers during the growing period, whereas plasma INS concentrations were similar between treatment groups during realimentation (Figure 3B). Plasma GH concentrations were similar between the

treatment groups at each serial harvest point (Figure 3C). Increased plasma NEFA and GH, and decreased INS concentrations along with lower BW, indicated that the GRW-FNSH steers were undergoing restricted growth during the growing period compared with the FNSH-FNSH steers.

Similar to plasma concentrations of INS, plasma leptin concentrations for FNSH-FNSH steers increased ($P < 0.001$) from d 0 to 88 and then plateaued, whereas plasma leptin concentrations increased linearly ($P < 0.001$) throughout in the GRW-FNSH steers (Figure 3D). Plasma leptin concentrations were higher ($P < 0.02$) for FNSH-FNSH steers compared with GRW-FNSH steers during the 116 d growing period, but were similar between treatment groups during the finishing period. As a fat cell increases in mass, peripheral concentrations of leptin have been reported to increase as well (Daniel et al., 2002; Delavaud et al., 2002; Geary et al., 2003). Additionally, steers undergoing restricted growth had much lower concentrations of plasma leptin than steers targeted to gain faster (Hersom et al., 2004). Plasma ghrelin concentrations for FNSH-FNSH steers increased quadraticly ($P < 0.001$) over time, whereas plasma ghrelin concentrations were unchanged for the GRW-FNSH steers over time (Figure 3E). Plasma ghrelin concentrations were similar for FNSH-FNSH and GRW-FNSH steers during the 116 d growing period. However, plasma ghrelin concentrations were higher ($P < 0.01$) for FNSH-FNSH steers compared with GRW-FNSH steers on d 209. Ghrelin is a recently identified peptide hormone that has been reported to influence DMI, energy expenditure, and fat deposition in other species (Tschöp et al., 2000; Wang et al., 2002). Ghrelin is synthesized by abomasal and ruminal tissues of cattle, however little is known regarding its role in beef cattle (Hayashida et al., 2001; Gentry et al., 2003). For steers in the FNSH-FNSH treatment, plasma leptin:ghrelin concentrations were increased on d 88 then decreased quadraticly ($P < 0.001$) to d 209, whereas GRW-FNSH plasma leptin:ghrelin concentrations increased linearly ($P < 0.001$) and peaked on d 165 of the finishing period (Figure 3F). Leptin:ghrelin was greater ($P < 0.10$) for FNSH-FNSH steers compared with the GRW-FNSH steers during the growing period, whereas the leptin:ghrelin concentration was increased ($P < 0.05$) for GRW-FNSH steers compared with FNSH-FNSH steers on d 209. Plasma leptin and ghrelin concentrations did fluctuate relative to nutritional status during the feeding period. However, increased plasma leptin and ghrelin concentrations predominately occurred in steers consuming the high-energy diet (FNSH-FNSH).

In conclusion, differential rate of gain the growing period resulted in differences in body composition of cattle. These differences are associated with differences in plasma leptin and ghrelin concentrations as well as other indices of metabolic status. Plasma ghrelin concentrations in cattle relative to body composition have not previously been reported, however, in other species, ghrelin has been reported to influences DMI, energy metabolism, and fat deposition. Thus, the role of ghrelin in beef cattle warrants further investigation as DMI, energy metabolism, and fat deposition contribute to the efficiency of production.

Table 3. Standard carcass characteristics at serial harvest points throughout the feeding period

	Serial Harvest Day					*P* value		
Variable [a]	d 88	d 116	d 165	d 209	Root MSE	TRT	HRV	TRT x HRV
Observations								
FNSH-FNSH [b]	8	8	8	7	---	---	---	---
GRW-FNSH [b]	7	8	8	8	---	---	---	---
HCW, lb								
FNSH-FNSH	554	612	709	798	39.9	0.001	0.001	0.22
GRW-FNSH	519	531	654	774				
REA, in^2								
FNSH-FNSH	10.2	10.6	11.5	11.5	0.92	0.32	0.001	0.46
GRW-FNSH	9.7	10.0	11.2	11.9				
RF, in								
FNSH-FNSH	0.35	0.34	0.38	0.57	0.11	0.001	0.001	0.28
GRW-FNSH	0.20	0.22	0.32	0.35				
MARB[c]								
FNSH-FNSH	381	408	470	729	79.7	0.001	0.001	0.001
GRW-FNSH	346	336	420	416				
YG								
FNSH-FNSH	2.8	2.7	2.9	3.7	0.48	0.001	0.001	0.46
GRW-FNSH	2.3	2.2	2.6	2.9				
KPH								
FNSH-FNSH	2.9	2.3	2.1	1.9	0.48	0.01	0.003	0.15
GRW-FNSH	2.1	1.8	1.9	1.9				
DP, %								
FNSH-FNSH	57.5	58.6	61.2	62.8	1.49	0.001	0.001	0.04
GRW-FNSH	57.2	55.4	59.1	59.9				

[a] HCW = Hot carcass weight, DP = dressing percent, REA = ribeye area measurement, RF = ribfat measurement, MARB = marbling score, and YG = calculated yield grade.

[b] TRT = FNSH-FNSH = steers receiving a high-energy diet during the entire feeding period(d 0-209), GRW-FNSH = steers receiving a low- energy diet during the growing period(d 0-116), followed by a high-energy diet during the finishing period (d 117-209).

[c] Marbling scale; practically devoid = 100, traces = 200, slight = 300, small = 400, modest = 500, moderate = 600, slightly abundant = 700, moderately abundant = 800, abundant = 900.

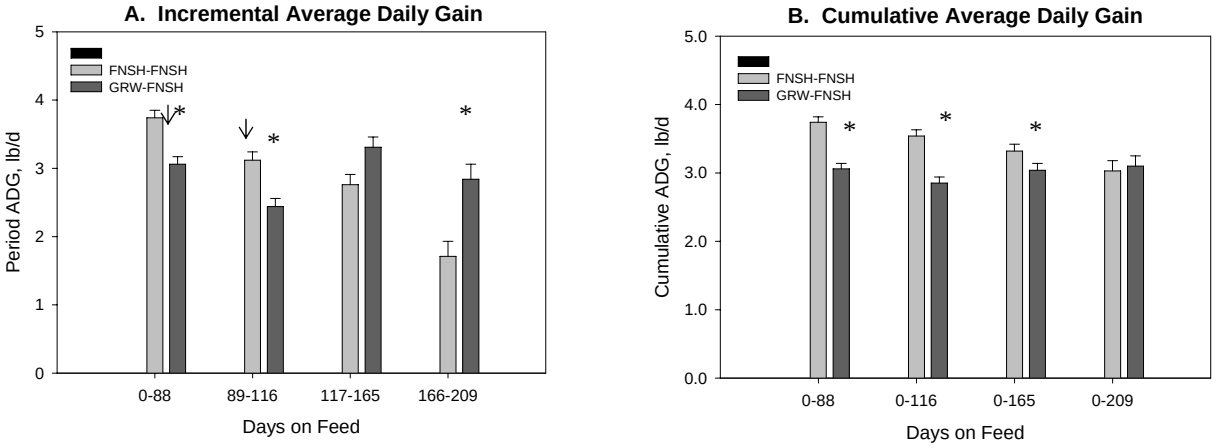

Figure 1**.** Performance characteristics, including incremental ADG (A) and cumulative ADG (B). FNSH-FNSH = steers receiving a high-energy diet during the entire feeding period (d 0-209), GRW-FNSH = steers receiving a low-energy diet during the growing period (d 0-116), followed by a high-energy diet during the finishing period (d 117-209). The first arrow indicates when energy content of the growing diet received by the GRW-FNSH steers was reduced further. The second arrow indicates when the GRW-FNSH steers were switched to the high-energy diet to begin the finishing period. * Within a harvest point, FNSH-FNSH vs. GRW-FNSH differ as a result of dietary treatment, $P < 0.05$.

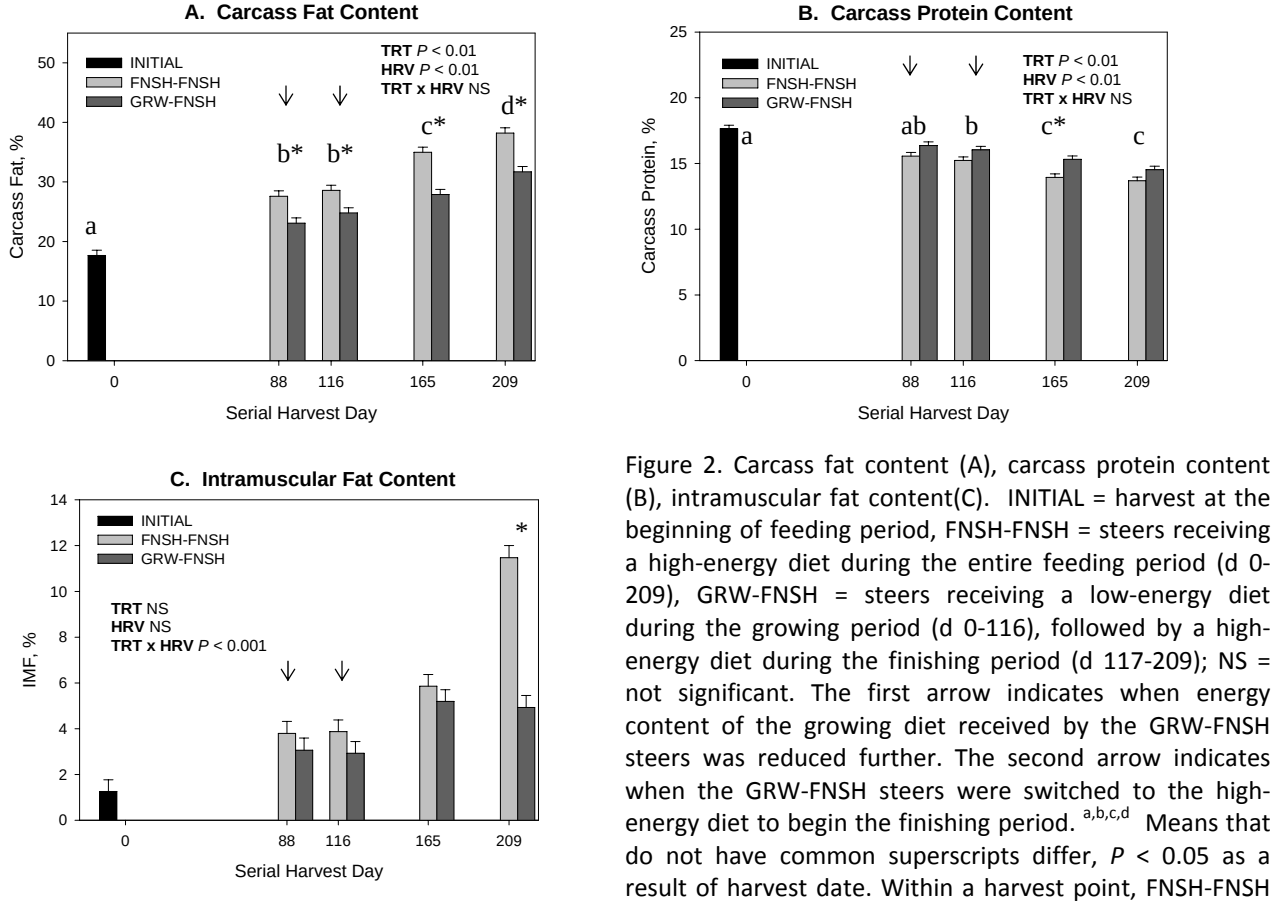

Figure 2. Carcass fat content (A), carcass protein content (B), intramuscular fat content(C). INITIAL = harvest at the beginning of feeding period, FNSH-FNSH = steers receiving a high-energy diet during the entire feeding period (d 0-209), GRW-FNSH = steers receiving a low-energy diet during the growing period (d 0-116), followed by a high-energy diet during the finishing period (d 117-209); NS = not significant. The first arrow indicates when energy content of the growing diet received by the GRW-FNSH steers was reduced further. The second arrow indicates when the GRW-FNSH steers were switched to the high-energy diet to begin the finishing period. ^{a,b,c,d} Means that do not have common superscripts differ, $P < 0.05$ as a result of harvest date. Within a harvest point, FNSH-FNSH vs. GRW-FNSH differ as a result of dietary treatment, *$P <$ 0.05.

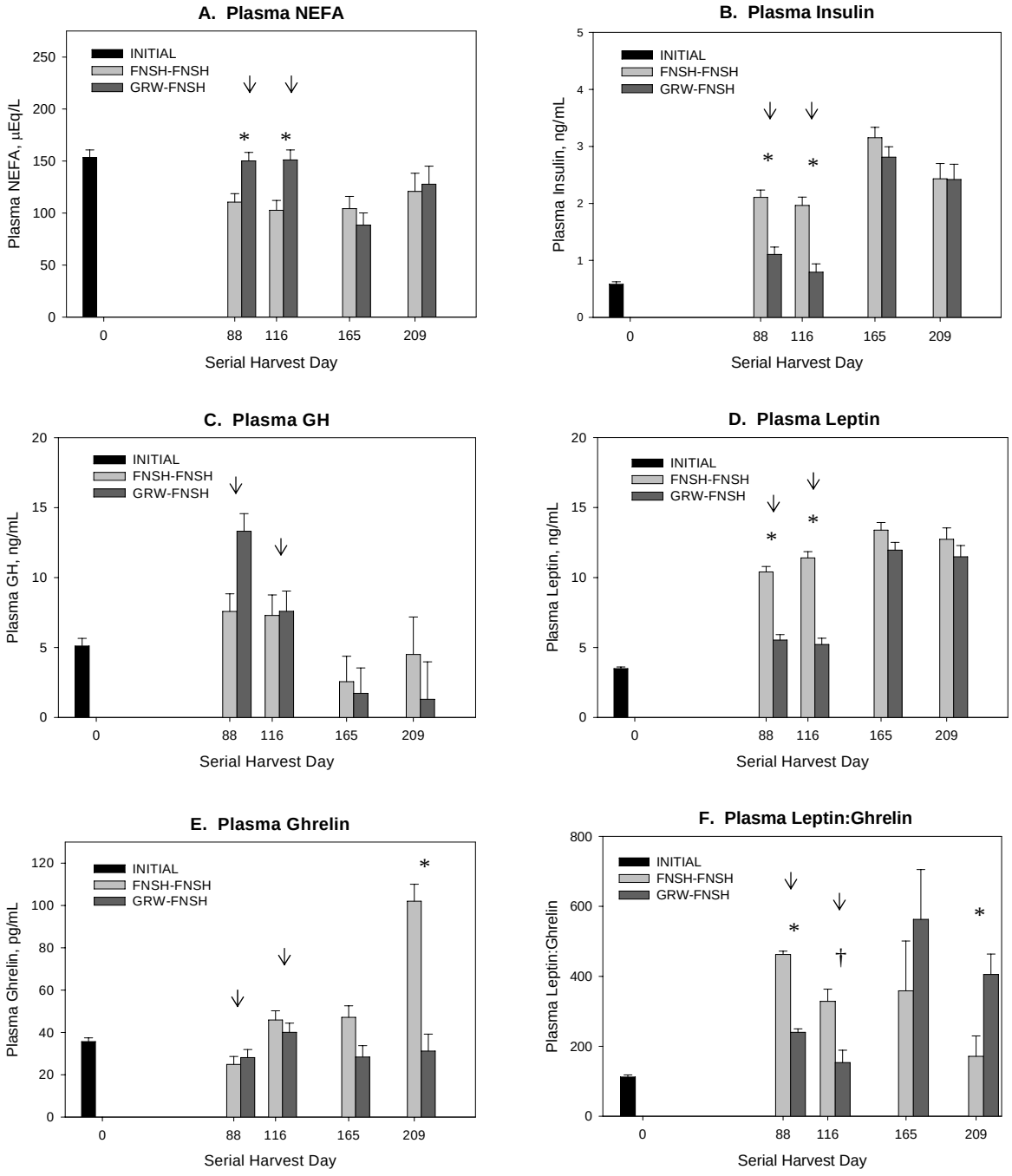

Figure 3. Plasma NEFA (A), insulin (B), GH (C), Leptin (D), Ghrelin (E), and Leptin:Ghrelin (F) concentrations. INITIAL = serial harvest at the beginning of feeding period, TRT = FNSH-FNSH = steers receiving a high-energy diet during the entire feeding period, GRW-FNSH = steers receiving a low-energy diet during the growing period, followed by a high-energy diet during the finishing period. The first arrow indicates when energy content of the growing diet received by the GRW-FNSH steers was reduced further. The second arrow indicates when the GRW-FNSH steers were switched to the high-energy diet to begin the finishing period. * Within a harvest point, FNSH-FNSH vs.GRW-FNSH differ as a result of dietary treatment, *$P < 0.05$ and † $P = 0.08$.

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