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Short Communication

Insulin resistance increases as days on feed advance in feedlot *Bos indicus* beef cattle offered a high-concentrate finishing diet

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ABSTRACT

This experiment evaluated the effects of advancing days on feed (DOF) on insulin resistance (IR) of finishing *Bos indicus* bulls offered a high-concentrate diet. On day 0, 100 *Bos indicus* Nellore bulls were housed in 12 feedlot pens (8 or 9 bulls/pen) for a 108-d feeding period, in a manner that all pens had an equivalent BW at the beginning of the finishing period. Bulls received the same diets throughout the 108-d feeding period. Diets were corn-based (38% starch) and were offered in amounts to ensure ad libitum intake. Individual shrunk BW was obtained on days 0 and 108 after 16 h of feed and water withdrawal, whereas unshrunk BW was recorded on days 19 and 60 for average daily gain (ADG) calculation. From days 0 to 108, feed intake and feed efficiency (FE) were calculated from each pen. Blood samples were collected on days 0, 60, and 108 to assess serum concentrations of haptoglobin, cortisol, glucose, and non-esterified fatty acids (NEFA), and insulin. Glucose, NEFA, and insulin concentrations were used to determine revised quantitative insulin sensitivity check index (RQUICKI), so that lower RQUICKI values indicate a greater IR of the herd. Pen was used as the experimental unit. Overall, DMI increased as DOF also increased (R^2 = 0.71), being greater from days 19 to 60 and 60 to 108 vs. 0 to 19 ($P < 0.0001$), but did not differ between days 19 to 60 and 60 to 108 ($P = 0.79$). Conversely, ADG and FE linearly decreased as DOF increased from days 0 to 108 ($P < 0.0001$; R^2 = 0.68 and 0.79, respectively). Log-transformed RQUICKI decreased as DOF increased up to 108 ($P < 0.0001$; $r = -0.61$). Similarly, serum concentrations of haptoglobin and cortisol increased as RQUICKI increased ($P < 0.0001$; r = 0.43 and 0.67, respectively). In summary, insulin resistance, per RQUICKI, increased and performance reduced in feedlot bulls with the advance of days on feed. Moreover, inflammatory markers were also positively associated with insulin resistance, suggesting that inflammation might be involved with the incidence of insulin resistance.

Lay Summary

This experiment was designed to evaluate the effects of feeding a high-starch during the feedlot phase (108 d) on performance, inflammatory markers, and insulin resistance of *Bos indicus* bulls. As feeding period increased, performance of the animals was reduced, whereas inflammatory markers were positively correlated with days on feed. Moreover, insulin resistance state also worsened as days on feeding increased, indicating that offering a high-starch diet for an extended period of time might lead to chronic inflammation and the occurrence of insulin resistance, which, in turn, could help us to explain the often observed decrease on performance of animals in the later stages of the feedlot phase.

Key words: beef cattle, feedlot, inflammation, insulin resistance, performance

Abbreviations: ADG, average daily gain; BW, body weight; DMI, dry mater intake; DOF, days on feed; FE, feed efficiency; G:F, gain to feed; IR, insulin resistance; NEFA, non-esterified fatty acids; RQUICKI, revised quantitative insulin sensitivity check index

Introduction

Insulin resistance (IR) has been commonly reported as a dairy cow syndrome, occurring either due to inadequate (Sinclair, 2010) or excessive (Leiva et al., 2015) nutrient intake in the post-partum and late-lactation period, respectively. However, we are unaware of other studies evaluating the effects of feeding a high-concentrate diet on IR of *Bos indicus* feedlot cattle. Moreover, non-esterified concentrations (NEFA) are often increased in insulin resistant animals (Leiva et al., 2014), which can also be associated with an inflammatory state of ruminants (Cooke and Bohnert, 2011). To the best of our knowledge, no other research effort evaluated possible correlations, if any, of IR occurrence and markers of inflammation in beef cattle.

Previous studies in the literature have reported that cattle fed high-concentrate diets often present a reduction in feed efficiency parameters (G:F or FE) as days on feed (DOF) increase, an effect primarily attributed to the increased energy required per unit of gain, as cattle approach mature body weight (BW; NASEM, 2016). Nonetheless, inclusion of high-lipid and high-fiber byproduct into the diet of feedlot cattle also reduced G:F (Joy et al., 2016) and insulin sensitivity reduced as DOF increased in heifers offered a high-concentrate diet for 160 d (Joy et al., 2017). However, Joy et al. (2017) fed the

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heifers individually and, consequently, intake pattern might differ, warranting further research efforts that can evaluate performance and the occurrence of IR in commercial feedlots where cattle are reared in collective pens. Based on this rationale, we hypothesized that offering a high-concentrate diet for *Bos indicus* feedlot cattle and reduce insulin sensitivity as DOF advances, due to increasing levels of inflammatory markers. Therefore, our objective was to evaluate the effects of feeding a high-concentrate diet for a 108-d period on performance, IR, and concentrations of inflammatory markers in feedlot cattle.

Experimental Procedure

This experiment was conducted at a commercial feedlot operation (Fazenda Flórida), located in Guaiçara, SP, Brazil (21°37′33″ S, 49°47′52″ W, and elevation of 437 m) from May to October 2020. All animals were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Nutricorp Institutional Animal Care and Use Committee (#007/2020).

Animals

On day −2 of the experiment, 100 *Bos indicus* Nellore bulls were individually weighed [initial body weight (BW) 341 ± 18.5 kg; initial age = 21 ± 2.3 mo] at the commercial cow-calf ranch (Agro Rondinha, Camapuã, MS, Brazil). Bulls were loaded into 2 commercial livestock trailers in a manner that both trailers left the cow-calf farm at the same time (1400 h), were transported for 880 km through the same route until arriving at a commercial feedlot plant (Guaiçara, SP, Brazil) in the evening of day −1. On the morning of day 0, bulls were individually ear-tagged, vaccinated against respiratory (5 mL/head; Bayovac Respiratória RD; Bayer SA, São Paulo, SP, Brazil) and clostridial (5 mL/head; Excell-10; Venco Saúde Animal, Londrina, PR, Brazil) pathogens, and administered an anthelmintic (1 mL/50 kg BW; Cydectin, Zoetis) for internal and external parasites. After initial processing on day 0, bulls were housed in 12 feedlot pens (8 or 9 bulls/pen) for a 108-d feeding period, in a manner that all pens had an equivalent BW at the beginning of the finishing period. Pens were unpaved (18 × 5 m and 1.0 m of linear feedbunk/bull). Bulls received the same diets throughout the 108-d feeding period. Diets were composed of ground flint corn (mean particle size = 1.62 mm), whole cottonseed, grass silage, DDGS, sugarcane bagasse, urea, mineral-vitamin mix, and water. The diet contained 38% starch, offered in amounts to ensure ad libitum intake, and yield 5% orts.

Sampling

Individual shrunk BW was obtained on days 0 and 108 after 16 h of feed and water withdrawal, whereas unshrunk BW was recorded on days 19 and 60 for average daily gain (ADG) determination. From days 0 to 108, feed intake (DM basis) was evaluated from each pen by collecting and weighing offered and non-consumed feed daily. Samples of offered and non-consumed feed were dried following the microwave technique for daily DM calculation (Oliveira et al., 2015). Feed intake of each pen was divided by the number of animals within each pen and expressed as kg per animal/day. Feed efficiency (FE) was calculated using total BW gain from days 0 to 19, 19 to 60, and 60 to 108. Prior to the morning feeding, blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing no additive for serum collection on days 0 (prior to feedlot entry), 60, and 108 of the experiment.

Laboratorial analyses and insulin resistance (IR) assessment

Blood samples were placed immediately on ice after collection, centrifuged (2,500 × *g* for 30 min; 4 °C) for serum harvest, and stored at −20 °C on the same day of collection. All samples were analyzed for serum concentrations of haptoglobin (Cooke and Arthington, 2013), cortisol (radioimmunoassay kit #07221106, MP Biomedicals, Santa Ana, CA), glucose, and non-esterified fatty acids (NEFA; Carysta High Volume Chemistry Analyzer; Zoetis), and insulin (PI-12K; Millipore Sigma, Burlington, MA). The intra- and inter-assay CV were 3.8% and 7.2% for haptoglobin, 5.6% and 6.1% for cortisol, and 8.6% and 8.5% for insulin. All glucose and NEFA samples were analyzed in a single assay with an intra-assay CV of < 5%.

For the IR determination, concentrations of glucose, NEFA, and insulin obtained prior to management (days 0 and 108) or feeding (day 60) were used to determine revised quantitative insulin sensitivity check index (RQUICKI). This methodology has been used to estimate insulin sensitivity in ruminants, which is an approach to assess insulin resistance according to the equation proposed by Perseghin et al. (2001): RQUICKI = 1/[log(glucose) + log(insulin) + log(NEFA)].

Statistical analysis

All data were analyzed using pen as the experimental unit, using the MIXED procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC), and Satterthwaite approximation to determine the denominator degrees of freedom for tests of fixed effects. Random statement for DMI and FE data included pen, whereas bull(pen) was used for ADG and physiological data. The specified term for repeated statements was day or period, with pen or bull(pen) as subjects. The covariance structure used was compound symmetry, which provided the smallest Akaike Information Criterion and hence the best fit for all variables analyzed herein. All RQUICKI data were tested for normality with Shapiro–Wilk and a log-transformation was needed prior to the analysis. Regression and correlation analysis were evaluated to determine possible relationships, if any, between IR, DOF, performance (DMI, ADG, and FE), as well as serum haptoglobin and cortisol concentrations. All results are reported as least square means with significance set at $P \leq 0.05$ and tendencies denoted if $P > 0.05$ and ≤ 0.10. Repeated measures are reported according to main treatment effect if no higher-order interactions were detected.

Results

Overall, DMI increased as DOF also increased, being greater from days 19 to 60 and 60 to 108 vs. 0 to 19 ($P < 0.0001$), whereas no differences were observed between days 19 to 60 and 60 to 108 ($P = 0.79$; Table 1). On the other hand, as DOF increased from days 0 to 108, ADG and FE linearly decreased ($P < 0.0001$; Table 1). When the values were plotted and analyzed, high *R*-squared values were obtained, such as 0.71, 0.68, and 0.79 for DMI, ADG, and FE, respectively.

In the present experiment, day effects ($P < 0.001$) were observed for glucose (69.2, 94.8, and 64.9 mg/dL on days 0, 60, and 108, respectively; SEM = 2.26), insulin (14.5, 50.7,

and 27.0 µIU/mL on days 0, 60, and 108, respectively; SEM = 3.24), and NEFA (0.267, 0.176, and 0.705 mEq/L on days 0, 60, and 108, respectively; SEM = 0.0302). Therefore, as DOF increased, log-transformed RQUICKI decreased ($P < 0.0001$; Pearson correlation coefficient = −0.61; Figure 1). Moreover, the dispersion of the RQUICKI values is reduced as the study progresses (Figure 1). Similarly, as RQUICKI increased, serum concentrations of haptoglobin (2.77, 2.61, and 2.15 µg/mL for days 0, 60, and 108, respectively) and cortisol (20.5, 31.2, and 30.5 ng/mL for days 0, 60, and 108, respectively) also increased on a linear fashion ($P < 0.0001$; Pearson correlation coefficient = 0.43 and 0.67, respectively).

Discussion

The main goal of the present experiment was to evaluate the effects of DOF in feedlot *Bos indicus* animals on insulin sensitivity, performance, and physiological parameters, such as serum haptoglobin and cortisol concentrations.

Table 1. Effects of days on feed (DOF) on performance of feedlot *Bos indicus* bulls receiving a high-starch diet (38% on a dry matter basis) for 108 d

Period	DMI,[1] kg/d	ADG,[1] kg	FE,[1] kg/kg
Days 0 to 19	6.71[b]	2.00[c]	0.297[c]
Days 19 to 60	9.63[a]	1.50[b]	0.157[b]
Days 60 to 108	9.71[a]	1.32[a]	0.136[a]
SEM	0.121	0.053	0.006
P	< 0.0001	< 0.0001	< 0.0001

[1]DMI, dry matter intake; ADG, average daily gain; FE, feed efficiency.
[a–c]Different letters indicate differences at $P < 0.05$ level.

Reduced insulin sensitivity and, consequently, increased resistance has been reported in late-lactating dairy cattle consuming excessive energy and, more specifically, starch (Leiva et al., 2015). In beef cattle, Joy et al. (2017) demonstrated that area under the curve of insulin linearly increased as DOF advanced in feedlot beef heifers fed a high-concentrate diet. Hence, a logical rationale would be to feed low-starch, high-byproduct diets to feedlot cattle. However, feeding a high-lipid, high-fiber byproduct pellet worsened heifer performance as study progressed until day 160 when compared with cohorts fed a high-corn diet (Joy et al., 2017). Therefore, additional studies are warranted to elucidate how insulin sensitivity is altered as DOF increases in feedlot beef cattle. The hypothesis of the present experiment was originated from the fact 1) that feedlot animals usually become less FE as DOF increase and 2) that nutrient partitioning into adipose tissue may not explain this theory in its full sense. In the present experiment, bulls were slaughtered at approximately 466 kg (data not shown), which is substantially less than the mature BW of *Bos indicus* animals (517 kg; Valadares Filho et al., 2016), but IR increased as DOF also increased. Hence, other mechanisms, besides adipose tissue accretion and mature BW might play a key role in reducing insulin sensitivity of feedlot cattle consuming a high-concentrate, corn-based diet for an extended period of time.

In the present experiment, ADG and FE linearly decreased as DOF increased up to 108 d in feedlot bulls consuming diets containing 38% starch. Joy et al. (2017) observed quadratic responses on ADG and FE over a 160-d experimental period, divided into 40-d evaluation periods, in *Bos taurus* beef heifers consuming feedlot diets containing approximately 43% starch. These authors also reported that apparent nutrient digestibility also increased as DOF increased, with greatest values obtained between days 81 and 120. However,

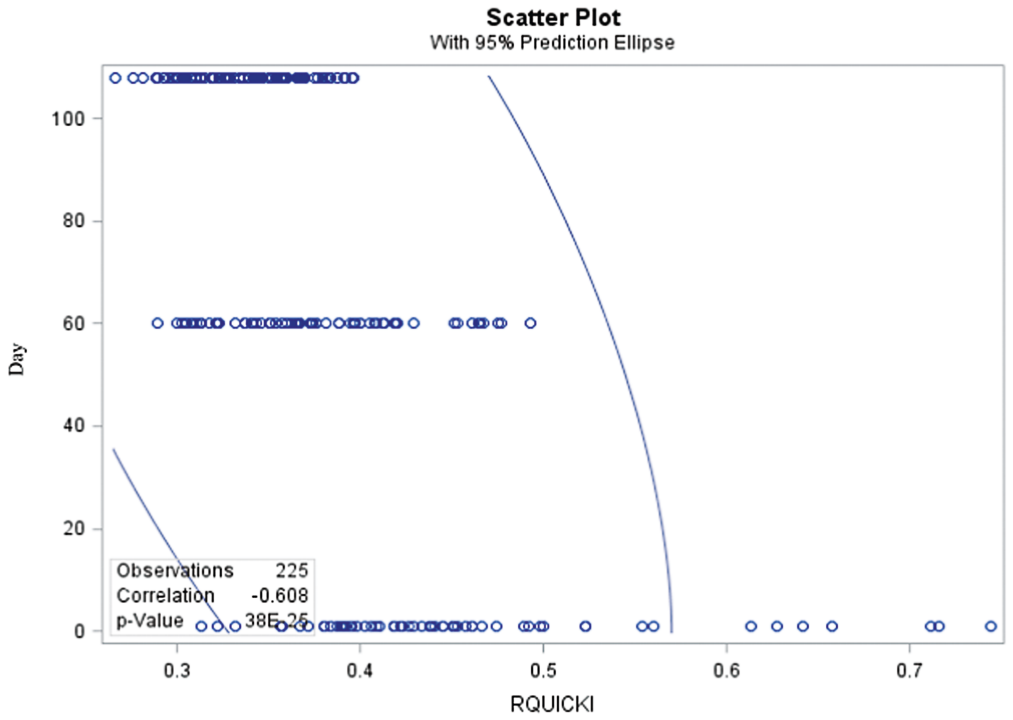

Figure 1. Pearson correlations between increasing days on feed (DOF) on insulin resistance measurement (RQUICKI).

no changes in plasma acetate and glucose clearance were observed, suggesting a possible effect of rumen function on occurrence of IR (Joy et al., 2017). One might speculate that the effects of inflammation during later stages of the productive cycle of feedlot cattle might also be playing a key role on rumen function and on the occurrence of decreased insulin sensitivity. In fact, Lippolis et al. (2017) demonstrated that beef steers challenged with lipopolysaccharide (LPS) had a greater haptoglobin and cortisol concentration, but reduced DM and neutral detergent fiber digestibility, suggesting a potential role of these inflammatory markers on rumen and gastrointestinal tract health and microbiome function. Lastly, it can be speculated that this shift in microbiome caused by a potential chronic inflammation might be predisposing the animals to rumen dysbiosis and occurrence of potential rumen health challenges, such as acidosis in the latter part of the feedlot (Castillo-Lopez et al., 2014). One might argue that LPS does not represent the same immune challenge as in the present trial, but it is noteworthy mention that LPS are also able to impact the integrity of intestinal cells, leading to leaky gut, and bacterial translocation into the systemic circulation, which, in turn, may start an inflammatory response in ruminants (Lippolis et al., 2017).

Corroborating these authors, our results demonstrate that as RQUICKI increased with advancing DOF, serum haptoglobin and cortisol concentrations also increased, suggesting that either 1) chronic inflammation might be causing IR or 2) the occurrence of IR in feedlot cattle offered a high-starch diet might be leading the animals into a chronic inflammatory state. In humans, pro-inflammatory cytokines released by the tissue resident macrophages can cause insulin resistance in adipose tissue, skeletal muscle, and liver by inhibiting insulin signaling (de Luca and Olefsky, 2008). Tissue macrophages are also able to produce leptin (Schneiderman et al., 2012) that, in turn, keep the concentration of inflammatory markers (i.e., haptoglobin and cortisol) elevated (Rodrigues et al., 2015) and potentially explaining the reduced performance observed herein. A clear connection between IR and acute/chronic inflammation, if any, has not been described in beef and dairy cattle (Leiva et al., 2014; Leiva et al., 2015; Joy et al., 2016; Joy et al., 2017), and our experiment is the first one reporting such potential association between inflammatory markers and IR. Nonetheless, more studies are warranted to evaluate these links in a more intensive trial.

In summary, increasing days on feed also increased insulin resistance and reduced performance of feedlot bulls offered a high-concentrate, corn-based diet. Moreover, inflammatory markers were also positively associated with the insulin resistance index used herein (RQUICKI), suggesting that inflammation might be playing a key role on this insulin resistant state. Therefore, more studies are warranted to understand potential paths of insulin resistance in feedlot beef animals and potential alternatives to alleviate such effects and to improve performance, health, and overall welfare of the beef cattle herd.

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Conflict of Interest Statement

The authors declare no real or perceived conflicts of interest.

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