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Effects of combining immunocastration and β-adrenergic agonists on the blood metabolites and their correlations with performance and carcass traits of finished Nellore cattle

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ABSTRACT - The objective of this study was to evaluate the effects of combining immunocastration and β-adrenergic agonists (β-AA) on blood metabolites and their correlations with performance and carcass traits of Nellore cattle. Ninety-six Nellore males were distributed in a randomized block design with a 2×3 factorial arrangement. The factors were two sexual conditions (immunocastrated and non-castrated), and three diets (control, with no inclusion of β-AA; RH, with 300 mg of ractopamine hydrochloride/d; and ZH, with 80 mg of zilpaterol hydrochloride/animal/d). The trial was conducted during 100 d, during which animals were fed ZH and RH for the last 30 and 33 d, respectively. Blood metabolites related to lipid and protein metabolism were assessed at the baseline (0 d of β-AA supplementation) and after 13 and 30 d of β-AA supplementation. No effect of sexual condition × β-AA supplementation × time on feed interaction was observed. Combining immunocastration with β-AA supplementation modified cholesterol and non-esterified fatty acids concentrations without affecting protein metabolism. Immunocastration enhances lipogenesis and reduces skeletal muscle accretion by increasing high-density lipoprotein and triglycerides concentrations and decreasing creatinine and creatine kinase concentrations, respectively. Zilpaterol hydrochloride enhances skeletal muscle accretion by decreasing urea and total protein concentrations and increases creatinine and creatine kinase concentrations without modifying lipid metabolism.

Keywords: animal production, physiology, ractopamine, testosterone, zilpaterol

1. Introduction

Castration is an alternative used worldwide to improve carcass fat deposition. However, surgical castration is associated with pain and harm to animal welfare (González et al., 2010; Gregory and Ford, 1983). Thus, due to increased public concern regarding animal welfare, some technologies, such as anti-gonadotropin-releasing factor vaccines, have been used as an animal-friendly alternative to surgical castration (Dunshea et al., 2005; Amatayakul-Chantler et al., 2012, 2013). Despite the benefits of castration on docility (Katz, 2007) and meat quality (Miguel et al., 2014; Gómez et al., 2017), bulls are more efficient in gaining muscle mass than steers, which is attributable to the effects of testosterone.

Greater production efficiency is mandatory in the modern beef cattle industry, and the use of feed additives, such as β-adrenergic agonists (β-AA), has demonstrated positive results on performance and meat production (Arp et al., 2014; Cônsolo et al., 2015; Mazon et al., 2019). β-adrenergic agonists are non-hormonal growth promoters that induce muscle hypertrophy in supplemented animals by increasing muscle protein synthesis and/or decreasing protein breakdown (Dunshea et al., 2005). Their use also induces lower carcass fat deposition by increasing lipolysis and decreasing lipogenesis (Dunshea et al., 2005).

Many studies have been conducted to evaluate the effects of immunocastration (Amatayakul-Chantler et al., 2012, 2013; Gómez et al., 2017; Miguel et al., 2014) and β-AA supplementation (Avendaño-Reyes et al., 2006; Arp et al., 2014; Cônsolo et al., 2015) in beef cattle. However, there is a lack of knowledge about how the combination of immunocastration and β-AA supplementation affects animal performance. In a previous study, Antonelo et al. (2017) reported that immunocastration allows for greater carcass fat deposition, whereas β-AA allows for greater carcass muscle deposition during the finishing period. Changes in growth rate and body gain composition caused by immunocastration and β-AA supplementation are closely correlated to different patterns of lipid and protein metabolism. Blood metabolite investigation may provide information about physiological changes and their relationship with animal performance and carcass traits.

Therefore, this study was carried out to evaluate the effects of combining immunocastration and β-adrenergic agonists on blood metabolites and their correlations with performance and carcass traits of finished Nellore cattle.

2. Material and Methods

The research was conducted in Pirassununga City, São Paulo, Brazil (21°59' S and 47°25' W parallels, at an altitude of 627 m). Research on animals was conducted according to the institutional committee on animal use (case number 13.1.541.74).

Ninety-six Nellore (*Bos indicus*) intact males (409±50 kg body weight [BW]; 20 months old) were used to evaluate the effects of immunocastration (non-castrated and immunocastrated) and β-AA (control with no β-AA; RH - ractopamine hydrochloride; ZH - zilpaterol hydrochloride) in a completely randomized block design experiment with a 2×3 factorial arrangement, with two blocks (light – 374±5.27 kg; heavy – 443±5.21) and eight treatment replications in each block, with a total of 16 replications per treatment. Animals in the immunocastrated group received two doses of immunocastration vaccine (Bopriva®, Zoetis Ltda, Campinas, SP, Brazil) within a 30-d interval, whereas the non-castrated animals were not immunized. The first group (n = 48), composed by 24 immunocastrated and 24 non-castrated animals, was housed in four pens (12 animals/pen) equipped with electronic gates (American Calan Inc., Northwood, NH, USA), which allowed individual control of feed intake. To avoid aggressiveness between immunocastrated and non-castrated animals in the same pen, they were divided by sex condition. Therefore, there were two pens with immunocastrated (12 animals each) and other two pens with non-castrated (12 animals each) animals. The second group (n = 48) was housed in individual pens that also allowed for individual feed intake measurement. Both facilities had covered feed bunks, concrete floors, and automatic waterers.

At the beginning of feedlot period, all animals were subjected to a 21-d adaptation period. Then, they were fed a total mixed common diet containing 760 g kg^{-1} of concentrate and 240 g kg^{-1} of roughage (Table 1) for 70 d (33 d before slaughter), after which two groups of animals received the β-AA diets, while those in the control group continued to receive the same diet. The ZH was administered at 80 mg/animal/d (Zilmax®, Merck Animal Health, São Paulo, Brazil), and RH was administered at 300 mg/animal/d (Optaflexx®, Elanco Animal Health, São Paulo, Brazil). The ZH diet was removed three days before slaughter according to the withdrawal period required by law for this product, whereas RH diet was removed one day before slaughter according to the required fasting period. Further information about experimental design, facilities, management and performance and carcass data are described in Antonelo et al. (2017), Brigida et al. (2018), and Mazon et al. (2019).

Blood samples were obtained by jugular venipuncture at the beginning of the feedlot period (0 d of feeding), at beginning of the β-AA supplementation (after 70 d of feeding), after 13 and 30 d of β-AA supplementation (83 and 100 d, respectively) to evaluate hemogasometry and biochemical profile.

For hemogasometry analyses, 3 mL of blood were collected in plastic syringes with no anticoagulant at 0 and 100 d of feeding. Shortly after the collection, few drops of blood were placed into an i-STAT EC8+® cartridge for reading the blood gas from a portable clinical analyzer (i-STAT®Co. – Abbott Laboratories, USA). Chloride, sodium, potassium, pH, pressure of carbon dioxide (PCO_2), and bicarbonate (HCO_3) were recorded. For biochemical profile analyzes, 4 mL of blood were collected in tubes vacutainer with no anticoagulant at 70, 83, and 100 d of feeding. Samples were centrifuged at 2000 × *g* for 20 min at 4 °C to separate the serum, which was frozen at −20 °C. Non-esterified fatty acids (NEFA), beta-hydroxybutyrate (BHBA), cholesterol, triglycerides, high-density lipoprotein (HDL), glucose, creatine kinase (CK), creatinine, urea, albumin, and total protein were analyzed with commercial kits (Randox Brazil LTDA, São Paulo, Brazil) in an RX Daytona Machine (Randox Laboratories Ltd., Crumlin, UK) at 37 °C. Intra- and interassay coefficient of variation threshold were considered to be 5 and 10%, respectively.

Simple descriptive statistics (PROC MEANS) were computed for performance rates, carcass traits, and retail cuts and trimming weights to characterize the animals. The blood metabolites data were evaluated as a randomized completely block design with a 2×3 factorial arrangement (sexual condition × β-AA supplementation) and were analyzed as time-repeated measurements using the MIXED procedure of SAS (Statistical Analysis System, version 9.4). The model included the fixed effects of sexual condition (immunocastrated and non-castrated), β-AA supplementation (control, RH, and ZH), time on feed, and their interactions. Blocks (initial BW) were considered as a random effect, and animal was considered the experimental unit. The covariance structures were modeled, and those best fitted for each trait were selected, based on the smallest value of corrected Akaike criteria (AICC) according to Wang and Goonewardene (2004). The least square means (LSMEANS) statement was used to calculate the adjusted means for treatment, and the means were compared by Student's t test. Differences were considered statistically significant when $P \leq 0.05$. Tendencies were considered when $0.10 \leq P < 0.05$. Additionally,

Table 1 - Dietary ingredients and chemical composition (DM basis) of the basal finishing diet

Item	Content
Ingredient (g kg^{-1})	
Corn silage	241.8
Corn grain	318.2
Soybean 45%	121.7
Citrus pulp pellet	300.0
Urea	12.2
Mineral mixture[1]	6.0
Chemical composition[2] (g kg^{-1})	
Dry matter (as-fed)	743.1
Crude protein	146.4
Rumen-degradable protein	99.3
Total digestible nutrients	745.3
Ether extract	30.0
Neutral detergent fiber	232.1
Acid detergent fiber	183.0
Ash	58.4

[1] The trace mineral mixture contained (per kg): zinc, 1715 mg; iron, 420 mg; calcium, 160 g; selenium, 6 mg; phosphorus, 17 g; manganese, 720 mg; magnesium, 14.3 g; copper, 360 mg; cobalt, 21.6 mg; iodine, 21.6 mg; sodium, 57 g; sulphur, 22.9 g; non-protein nitrogen, 395 g; monensin sodium, 714.3 mg; vitamin A, 71,500 IU; vitamin D3, 8940 IU; vitamin E, 208 IU.
[2] Diets were formulated to meet or exceed all nutrient requirement of finishing bulls (NRC, 2000).

Spearman correlation coefficients (PROC CORR) were generated to determine the relationship of blood metabolites to the performance and carcass traits.

3. Results

Means, standard deviations (SD), and minimum/maximum values for performance and carcass traits used to characterize the animals are presented in Table 2.

No significant sexual condition × β-AA supplementation × time on feed interactions or sexual condition × time on feed interactions were observed for any trait. An interaction between sexual condition and β-AA supplementation was observed for the NEFA concentrations (P = 0.014; Table 3). Immunocastrated animals fed ZH had lower NEFA concentrations than immunocastrated animals fed

Table 2 - Descriptive statistics for performance rates, carcass traits, and retail cuts of *Bos indicus* (Nellore) cattle

Characteristic	N	Mean	SD	Minimum	Maximum
Initial BW (kg)	96	410	50.0	300	538
BW at 70 d of feeding (kg)	96	502	52.2	394	632
Slaughter weight (kg)	96	539	53.9	436	668
ADG (kg/d; 70-100 d)	96	1.18	0.455	0.13	2.60
DMI (kg/d; 70-100 d)	96	9.2	1.73	5.3	15.4
G:F (ADG/DMI; 70-100 d)	96	0.13	0.049	0.02	0.26
Longissimus muscle area (cm^2)	96	65.5	9.41	50.4	89.1
Backfat thickness (mm)	96	4.0	1.19	1.9	8.6
Hot carcass weight (kg)	96	315	34.1	244	398
Dressing (%)	96	58.5	1.52	55.1	63.1
Forequarter (kg)	96	61.4	6.91	48.9	79.9
Hindquarter (kg)	96	70.3	7.43	51.1	86.4
Brisket, short ribs, and flank (kg)	96	3.0	3.05	16.3	30.4
Forequarter (kg of retail cuts)	96	47.2	5.60	34.6	60.7
Hindquarter (kg of retail cuts)	96	55.8	6.22	42.0	69.8
Total retail cuts (kg)	96	102.9	11.04	79.5	127.4
Forequarter fat trim (kg)	96	4.0	3.17	0.3	9.4
Hindquarter fat trim (kg)	96	2.7	0.98	0.4	6.9
Total fat trim (kg)	96	6.7	3.51	1.2	15.3

BW - body weight; ADG - average daily gain; DMI - dry matter intake; G:F - gain to feed ratio; SD - standard deviation.

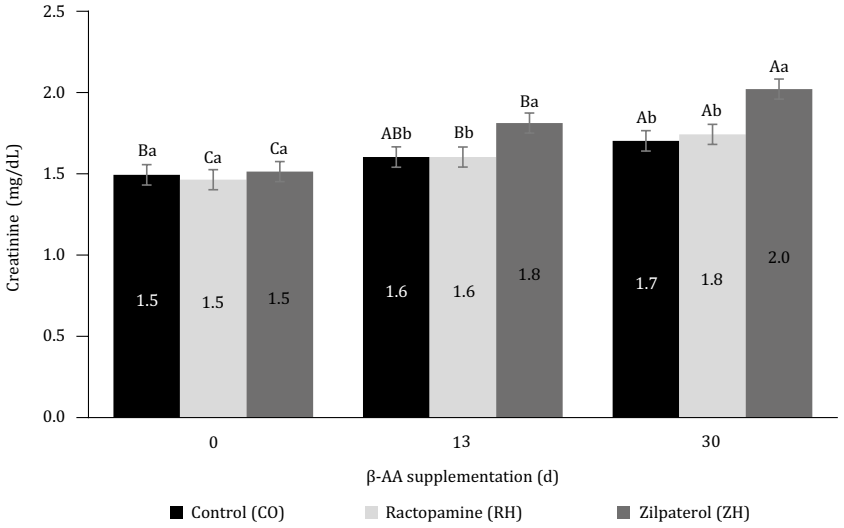
Table 3 - Effect of sexual condition[1] and diet[2] interaction on biochemical blood profile[3] of feedlot *Bos indicus* (Nellore) cattle

Characteristic	Immunocastrated			Non-castrated			SEM	P-value
	Control	RH	ZH	Control	RH	ZH		
Albumin (g/dL)	3.3Aa	3.4Aa	3.3Aa	3.2Aa	3.1Ba	3.1Aa	0.04	0.062
Cholesterol (mg/dL)	185Aa	180Aa	197Aa	158Ba	171Aa	165Ba	7.6	0.063
NEFA (mmol/L)	0.34Aa	0.36Aa	0.26Ab	0.29Ba	0.23Ba	0.28Aa	0.026	0.014

NEFA - non-esterified fatty acids; SEM - standard error of the mean.
[1] Immunocastrated (Bopriva®, Zoetis Industry Veterinary Products LTDA, São Paulo, SP, Brazil) males; non-castrated males.
[2] Control - with no inclusion of β-adrenergic agonists (β-AA); RH - ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN, USA), 300 mg/d; ZH - zilpaterol hydrochloride (MSD Animal Health, São Paulo, SP, Brazil), 80 mg/d.
[3] Average values for the all samples taken (0, 13, and 30 d of β-AA supplementation).
a,b - Values within a sexual condition with different lowercase letters differ significantly at P<0.10.
A,B - Values within a diet with different uppercase letters differ significantly at P<0.10.

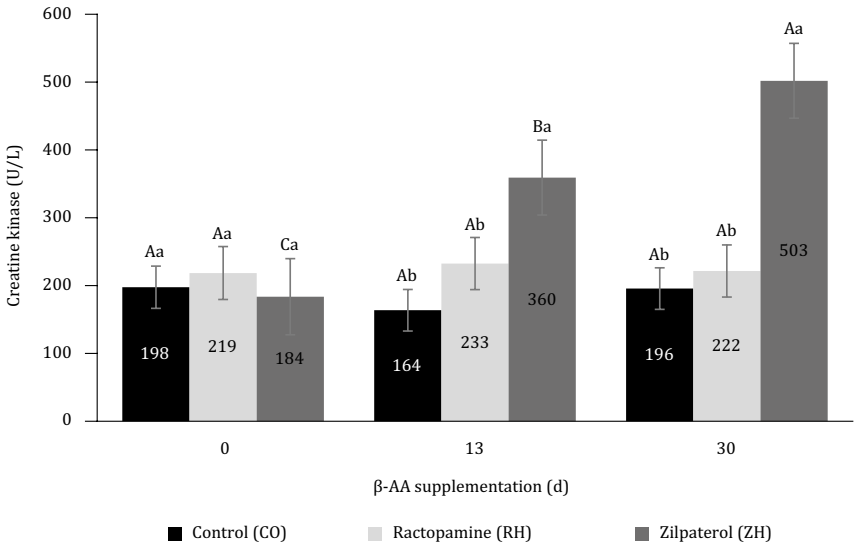
the control and RH diets (P<0.05), while non-castrated animals fed control and RH diets had lower NEFA concentrations than immunocastrated animals fed control and RH diets (P<0.05), respectively. Moreover, immunocastrated animals fed RH tended to have a higher albumin concentration (P = 0.062) than non-castrated animals fed RH. Non-castrated animals fed control and ZH tended to have a lower cholesterol concentration (P = 0.063) than immunocastrated animals fed control diet and ZH.

There was a β-AA supplementation × time on feed interaction for serum creatinine (Figure 1; P<0.001) and CK (Figure 2; P = 0.021) concentrations. The serum creatinine and CK concentrations did not



β-AA - β-adrenergic agonists.
Control - with no inclusion of β-AA; ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN, USA), 300 mg/d; zilpaterol hydrochloride (MSD Animal Health, São Paulo, São Paulo, Brazil), 80 mg/d.
a,b - Different letters within days of β-AA supplementation across treatment differ (P<0.05).
A,B,C - Different letters within treatment across days of β-AA supplementation differ (P<0.05).

Figure 1 - Serum creatinine concentration.



β-AA - β-adrenergic agonists.
Control - with no inclusion of β-AA; ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN, USA), 300 mg/d; zilpaterol hydrochloride (MSD Animal Health, São Paulo, São Paulo, Brazil), 80 mg/d.
a,b - Different letters within days of β-AA supplementation across treatment differ (P<0.05).
A,B,C - Different letters within treatment across days of β-AA supplementation differ (P<0.05).

Figure 2 - Serum creatine kinase concentration.

differ among the treatments at 0 d of β-AA supplementation (P>0.05), while at 13 and 30 d of β-AA supplementation, the animals fed ZH had higher serum creatinine and CK concentrations than those fed RH and control diets (P<0.05). In addition, serum creatinine concentrations increased for all the treatments over 30 d of feeding (P<0.05), while serum CK concentrations increased by 173% over 30 d of ZH feeding (P<0.001).

The immunocastrated animals had higher chlorine concentrations (P = 0.008) than non-castrated animals, without affecting other hemogasometry and electrolyte traits (Table 4). Moreover, RH and ZH tended to decrease sodium concentrations (P = 0.066) in comparison with control diet, but did not affect other hemogasometry and electrolyte traits.

Regarding the sexual condition, immunocastrated animals had higher HDL (P<0.001) and triglycerides (P<0.001) concentrations and lower CK (P = 0.007) concentrations and tended to have lower creatinine (P = 0.056) concentrations than non-castrated animals, with no changes for BHBA, glucose, serum urea, and total protein concentrations (Table 5). Regarding the β-AA supplementation, animals fed ZH had lower serum urea (P = 0.010) and total protein (P = 0.019) concentrations than animals fed RH and control diets. Animals fed ZH also had lower serum glucose (P = 0.003) concentrations

Table 4 - Effect of sexual condition[1] and diet[2] on hemogasometry[3] of feedlot *Bos indicus* (Nellore) cattle

Characteristic	Sexual condition (SC)		SEM	Diet (D)			SEM	P-value		
	ImC	NoC		Control	RH	ZH		SC	D	SC*D
pH	7.44	7.43	0.008	7.43	7.43	7.45	0.009	0.370	0.360	0.891
Chlorine (mEq/L)	102.0	101.2	0.38	101.6	101.4	101.6	0.41	0.008	0.845	0.896
Sodium (mEq/L)	139.5	139.2	0.46	139.8a	139.20b	139.0b	0.48	0.289	0.066	0.400
Potassium (mEq/L)	4.0	4.1	0.04	4.0	4.0	4.1	0.05	0.283	0.411	0.354
PCO_2 (mmHg)	40.8	41.6	1.30	39.7	42.7	41.2	1.44	0.513	0.126	0.553
HCO_3 (mEq/L)	27.4	27.5	0.35	27.0	27.6	27.8	0.43	0.709	0.399	0.169
PCO_2:HCO_3 ratio	1.51	1.54	0.060	1.50	1.56	1.50	0.065	0.586	0.442	0.192

PCO_2 - carbon dioxide pressure; HCO_3 - bicarbonate; SEM - standard error of the mean.
[1] ImC - immunocastrated (Bopriva®, Zoetis Industry Veterinary Products LTDA, São Paulo, SP, Brazil) males; NoC - non-castrated males.
[2] Control - with no inclusion of β-adrenergic agonists (β-AA); RH - ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN, USA), 300 mg/d; ZH - zilpaterol hydrochloride (MSD Animal Health, São Paulo, SP, Brazil), 80 mg/d.
[3] Average values for the all samples taken (0 and 100 d of feeding).
a,b - Values within a row with different letters differ significantly at P<0.10.

Table 5 - Effect of sexual condition[1] and diet[2] on biochemical blood profile[3] of feedlot *Bos indicus* (Nellore) cattle

Characteristic	Sexual condition (SC)		SEM	Diet (D)			SEM	P-value		
	ImC	NoC		CO	RH	ZH		SC	D	SC*D
BHBA (mmol/L)	2.2	2.3	0.17	2.1b	2.4a	2.2ab	0.18	0.529	0.025	0.813
HDL (mg/dL)	2.1	1.8	0.07	1.9	2.0	2.0	0.07	<0.001	0.364	0.555
Triglycerides (mg/dL)	24.0	20.4	1.02	21.0	22.5	23.1	1.11	<0.001	0.144	0.467
Glucose (mg/dL)	83.0	80.3	1.71	85.5a	81.9ab	77.6b	1.95	0.148	0.003	0.126
Urea (mg/dL)	42.0	42.1	0.66	42.4a	43.4a	40.5b	0.78	0.876	0.010	0.117
Creatinine (mg/dL)	1.6	1.7	0.05	-	-	-	-	0.056	-	0.155
Total protein (g/dL)	7.0	7.0	0.06	7.0a	7.1a	6.9b	0.07	0.416	0.019	0.485
Creatine kinase (U/L)	214	292	20.2	-	-	-	-	0.007	-	0.488

BHBA - beta-hydroxybutyrate; HDL - high-density lipoprotein; SEM - standard error of the mean.
[1] ImC - immunocastrated (Bopriva®, Zoetis Industry Veterinary Products LTDA, São Paulo, SP, Brazil) males; NoC - non-castrated males.
[2] Control - with no inclusion of β-adrenergic agonists (β-AA); RH - ractopamine hydrochloride (Elanco Animal Health, Greenfield, IN, USA), 300 mg/d; ZH - zilpaterol hydrochloride (MSD Animal Health, São Paulo, SP, Brazil), 80 mg/d.
[3] Average values for the all samples taken (0, 13, and 30 d of β-agonist adrenergic supplementation).
a,b - Values within a row with different letters differ significantly at P<0.05.

than animals fed the control, which did not differ between other treatments. Moreover, animals fed RH showed higher BHBA (P = 0.024) concentrations than animals fed the control diet, with no differences between other treatments.

Creatinine, CK, and triglycerides were the metabolites most correlated (P<0.05) with performance and carcass traits (Table 6). On the other hand, urea and NEFA had no correlation (P>0.05) with any trait evaluated. Moreover, albumin was negatively correlated with CK (r = −0.21) and positively correlated with triglycerides (r = 0.23) and cholesterol (r = 0.22), which were positively correlated with each other (r = 0.28; Table 7).

Table 6 - Simple correlation of performance, carcass traits, retail cuts, and biochemical blood profile of feedlot *Bos indicus* (Nellore) cattle

Characteristic	Urea	CREA	ALB	CK	HDL	BHBA	NEFA	TP	TRIG	GLUC	CHOL
Slaughter weight (kg)	0.02	0.20	−0.24*	0.23*	−0.14	−0.10	−0.13	0.18	0.17	−0.06	0.20
ADG (kg/d; 70-100 d)	−0.11	0.02	−0.05	0.27*	0.27*	0.20	−0.10	0.16	−0.06	−0.19	0.20*
DMI (kg/d; 70-100 d)	0.10	−0.27*	0.10	0.01	0.40**	0.02	0.02	0.24*	−0.09	0.21*	0.17
G:F (ADG/DMI; 70-100 d)	−0.18	0.07	−0.05	0.28*	0.15	0.25*	−0. 10	0.18	0.00	−0.31*	0.18
Longissimus muscle area (cm^2)	−0.15	0.22*	−0.19	0.32*	−0.16	−0.06	−0.14	0.03	0.22*	−0.08	0.07
Backfat thickness (mm)	0.10	−0.11	0.08	0.04	0.05	0.00	−0.03	−0.08	−0.10	−0.19	−0.01
Hot carcass weight (kg)	0.01	0.27*	−0.22*	0.28*	−0.13	−0.06	−0.12	0.17	0.20	−0.06	0.21*
Dressing (%)	−0.04	0.37*	0.04	0.29*	−0.02	0.14	0.00	0.03	0.18	0.01	0.10
Forequarter (kg)	0.03	0.31*	−0.23*	0.22*	−0.13	−0.07	−0.12	0.20	0.22*	−0.07	0.17
Hindquarter (kg)	−0.07	0.30*	−0.18	0.31*	−0.11	−0.03	−0.10	0.11	0.29*	−0.06	0.21*
Brisket, short ribs, and flank (kg)	0.03	0.07	−0.22*	0.22*	−0.03	−0.16	−0.11	0.10	0.21*	−0.02	0.23*
Forequarter (kg of retail cuts)	0.05	0.26*	−0.15	0.30*	−0.01	0.16	−0.16	0.13	0.04	−0.17	0.03
Hindquarter (kg of retail cuts)	−0.06	0.31*	−0.18	0.34*	−0.11	−0.03	−0.12	0.10	0.29*	−0.10	0.23*
Total retail cuts (kg)	−0.01	0.31*	−0.17	0.35*	−0.07	0.06	−0.15	0.12	0.18	−0.15	0.14
Forequarter fat trim (kg)	0.02	0.16	−0.17	−0.07	−0.22*	−0.42*	0.01	0.16	0.37*	0.16	0.31*
Hindquarter fat trim (kg)	−0.03	0.09	0.06	0.02	0.09	−0.01	0.06	0.16	0.21*	0.05	0.14
Total fat trim (kg)	0.01	0.17	−0.14	−0.06	−0.18	−0.38*	0.03	0.19	0.40*	0.16	0.32*

ADG - average daily gain; DMI - dry matter intake; G:F - gain to feed; CREA - creatinine; ALB - albumin; CK - creatine kinase; HDL - high-density lipoprotein; BHBA - beta-hydroxybutyrate; NEFA - non-esterified fatty acid; TP - total protein; TRIG - triglycerides; GLUC - glucose; CHOL - cholesterol.
* P<0.05; ** P<0.01.

Table 7 - Simple correlation of biochemical blood profile of feedlot *Bos indicus* (Nellore) cattle

Characteristic	Urea	CREA	ALB	CK	HDL	BHBA	NEFA	TP	TRIG	GLUC	CHOL
Urea	-	0.21*	−0.07	−0.02	0.06	0.03	−0.20	−0.04	−0.07	−0.06	0.08
CREA		-	−0.07	0.08	−0.37*	0.04	−0.15	−0.13	0.20	−0.13	0.11
ALB			-	−0.21*	0.29*	0.20	0.14	0.14	0.23*	0.01	0.22*
CK				-	−0.07	0.03	−0.14	−0.18	0.13	−0.16	−0.04*
HDL					-	0.20	0.15	0.07	0.10	0.06	0.51
BHBA						-	0.24*	0.08	−0.10	−0.02	−0.06
NEFA							-	0.03	−0.02	0.36*	0.10
TP								-	0.13	0.05	0.13
TRIG									-	0.09	0.28*
GLUC										-	0.03
CHOL											-

CREA - creatinine; ALB - albumin; CK - creatine kinase; HDL - high-density lipoprotein; BHBA - beta-hydroxybutyrate; NEFA - non-esterified fatty acid; TP - total protein; TRIG - triglycerides; GLUC - glucose; CHOL - cholesterol.
* P<0.05.

4. Discussion

4.1. Immunocastration × β-AA supplementation interaction

Non-castrated animals fed RH tend to have lower albumin concentration than immunocastrated animals fed RH. Lower serum albumin concentration has been associated with increased animal feed efficiency (Paula et al., 2013). In this study, albumin was negatively correlated with slaughter, hot carcass, forequarter, brisket, short ribs, and flank weights, which are the main indicators of muscle deposition. Although albumin has a negative correlation with CK, which favors muscle deposition (Kaneko et al., 2008), the albumin difference found in this study may not be interpreted as a difference in muscle deposition because the main markers of the total amount of muscle mass (CK and creatinine) did not differ. Serum albumin also regulates the colloidal osmotic pressure of blood and binds and transports fatty acids, cholesterol, and some ions (copper, zinc, and calcium) during blood circulation (Evans, 2002). Thus, in this study, the increase of serum albumin in immunocastrated animals may be associated with the higher lipid metabolism in these animals than in non-castrated animals, regardless of the diet, as observed by the positive correlation between serum albumin and HDL, triglycerides, and cholesterol.

Regarding the effects of sexual condition and β-AA supplementation on lipid metabolism, non-castrated animals fed control and ZH tend to have lower serum cholesterol concentrations than immunocastrated animals fed control and ZH, respectively, which may be related to the testosterone effect (Dunshea et al., 2005; Kaneko et al., 2008), because there was no effect of β-AA supplementation on cholesterol concentration within each sexual condition. Cai et al. (2015) observed that castration significantly increased the serum levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides of pigs fed a high-fat and high-cholesterol diet, which suggests that testosterone deficiency is associated with increased serum cholesterol levels and with cholesterol metabolism.

Some studies have reported an increase in the serum NEFA concentration in response to β-AA supplementation (Eisemann et al., 1988; Eisemann and Bristol, 1998), which may indicate the mobilization of fat stores (greater lipolysis) to provide energy support for the physiological functions of other tissues (Bryant et al., 2010). However, in this study, immunocastrated animals fed ZH had lower serum NEFA concentrations than immunocastrated animals fed control and RH. Moreover, immunocastrated animals fed control and RH had higher serum NEFA concentrations than non-castrated animals fed control and RH diets. These results suggest that muscle tissue may be using NEFA as an energy source in response to the ZH- and testosterone-promoted greater muscle growth. Cônsolo et al. (2015) also reported a lower serum NEFA concentration in nonimplanted Nellore heifers fed ZH than in those fed control due to an increase in the energy demands for muscle growth in ZH-supplemented animals. Thus, although the NEFA concentrations of all treatments were below 0.4 mmol/L, which would indicate healthy animals with a positive energy balance, the likely use of NEFA as an energy source may suggest that the concentration was above 0.4 mmol/L, which would indicate adipose tissue lipolysis (Miksa et al., 2004).

Despite both non-castrated and ZH-fed animals having a known impact on protein and lipid metabolism due to an increase in the energy demands for muscle growth, combining immunocastration with β-AA supplementation did not have enough impact on animal metabolism to promote significant changes in animal performance and carcass traits, as reported by Antonelo et al. (2017) and Brigida et al. (2018). Therefore, the effects of immunocastration with β-AA supplementation on animal metabolism were also evaluated separately.

4.2. Immunocastration effects

In the present study, immunocastration produced no changes in the blood electrolyte balance, which could be due to the lack of difference in water intake behavior (data not shown) between treatments.

In addition, these results may be supported by the absence of differences in the blood concentrations of HCO_3 and PCO_2 or their ratio, which is a major mechanism for maintaining the blood pH (Kaneko et al., 2008). However, hemogasometry analysis revealed that blood chloride concentration was changed by sexual condition, but this change had no representative effect on acid-basic balance, since sodium and potassium remain unchanged (Kaneko et al., 2008). Furthermore, the values were within the accepted normal range for beef cattle (97-111 mEq/L; Kaneko et al., 2008).

Several researchers have reported that immunocastration increased fat deposition in the carcass of beef cattle (Dunshea et al., 2005; Amatayakul-Chantler et al., 2012, 2013; Gómez et al., 2017). These results were also observed in this study, in which immunocastrated animals had greater serum HDL and triglycerides concentrations than non-castrated animals. The transport of triglycerides to target tissues occurs via chylomicrons that, upon reaching the tissue, are broken down by the lipoprotein lipase enzyme into glycerol and fatty acids to be absorbed and stored by the cells (adipose tissue) or oxidized (muscles; Nelson and Cox, 2012). The increase in serum triglycerides in immunocastrated animals may indicate a high activity of the lipoprotein lipase enzyme, thereby enhancing lipogenesis and accumulation of adipose tissue, as suggested in this study, by the positive correlation between triglycerides and total fat trim.

Immunocastration has been also related to the lower muscle mass deposition (Dunshea et al., 2005; Walker et al., 2010; Amatayakul-Chantler et al., 2013; Gómez et al., 2017). The results observed in this study are in a good agreement with this statement, since immunocastrated animals presented a lower serum creatinine and CK concentration than non-castrated animals. Creatinine is derived from the catabolism of creatine and phosphocreatine, molecules mainly contained in muscle, and is proposed as a marker for muscle mass in a steady state (Rennie and Millward, 1983; Virgili et al., 1994). Blood creatinine concentration has been positively associated with lean muscle in sheep (Cameron, 1992; Clarke et al., 1996) and greater muscle mass in bulls (Morgan et al., 1993). Moreover, the increase of serum CK concentrations indicate a muscle damage generated from an increased long-term physical stress due to an increase in muscle deposition. According to Kaneko et al. (2008), when energy muscle demand is not enough and more energy is required for muscle contraction, CK catalyzes the transfer of the adenosine diphosphate to form adenosine triphosphate, thereby providing energy for muscle. Furthermore, CK phosphorylates the creatinine in creatine-phosphate, which is an energy storage molecule present mainly in the skeletal muscles (Kaneko et al., 2008). The positive correlations found in this study between creatinine and CK with some indicators of muscle deposition, such as *Longissimus* muscle area, hot carcass weight, dressing percentage, and weight of total retail cuts are in a good agreement with those statements.

4.3. Effects of β-AA supplementation

Despite the tendency of animals fed ZH diet to have a lower sodium concentration than animals fed the control diet, the blood electrolyte balance was maintained and the blood pH was not changed. According to Freitas et al. (2010), the knowledge of blood electrolyte balance and its physiology and regulation is relevant because the health of the animal depends directly on the normal composition of fluid in body compartments. Therefore, the hemogasometry analysis revealed that β-AA supplementation did not affect the acid-base balance of feedlot Nellore cattle. Similarly, Abney et al. (2007) and Frese et al. (2016) also reported no difference in the hemogasometry of feedlot cattle fed β-AA diets.

β-adrenergic agonists are natural analogs of catecholamines; thus, they have glycogenolytic activity (Dunshea et al., 2005). In this study, animals fed ZH had lower serum glucose concentrations than animals fed the control diet, which may be associated with a higher energy demand proportioned by a greater muscle deposition in ZH animals. Cônsolo et al. (2015) also reported lower serum glucose concentrations in ZH-fed animals than in control animals. In this study, glucose concentration was negatively correlated with feed efficiency, which is in a good agreement with the fact that animals fed ZH have been associated with a higher performance in feedlot than non-supplemented animals (Arp et al., 2014; Cônsolo et al., 2015; Antonelo et al., 2017). Parr et al. (2011) also reported a higher

performance in ZH-fed animals, which was related to an increase in insulin-like growth factor-1 and NEFA concentration in ZH-fed animals than in control animals (Parr et al., 2014). In this study, glucose was positively correlated with NEFA, which was positively correlated with BHBA, a ketone body produced from the metabolism of NEFA by the liver that is usually elevated in states of negative energy balance (Ospina et al., 2010). However, in this study, RH-fed animals had higher BHBA concentrations than control animals without differences in glucose concentration. Thus, in spite of the lack of effect of β-AA supplementation on lipolysis, these results indicated that animals fed RH were not able to metabolize BHBA at sufficient rates to avoid accumulation in the blood, likely due to a poor adaptation to a negative energy balance (Herdt, 2000). Eisemann and Bristol (1998) and Van Bibber-Krueger et al. (2015) observed no changes in BHBA concentrations when clembuterol or ZH were administered to finishing steers, respectively.

It has been reported that the use of β-AA in cattle diet promotes greater muscle hypertrophy (Dunshea et al., 2005; Avendaño-Reyes et al., 2006). The results of the present study are in a good agreement with this statement, because animals fed ZH diet had a higher serum creatinine and CK concentration, which are good markers for muscle deposition (Virgili et al., 1994; Kaneko et al., 2008), than animals fed the control diet over 30 d of β-AA supplementation. Moreover, animals fed ZH diet had a lower serum urea and total protein concentration than animals fed RH and control diets. Those results were also reported by Parr et al. (2014) and Van Bibber-Krueger et al. (2015), which may reflect a decrease in protein catabolism in skeletal muscle (Van Bibber-Krueger et al., 2015) or an increase in tissue nitrogen deposition (Brake et al., 2011). These differences in blood metabolites related to muscle deposition between RH and ZH may be caused by differences in the β receptor, which is coupled to provide a cellular β-AA effect. The RH binds to a β1 receptor, and ZH binds to a β2 receptor (Mersmann, 1998). It has already been reported that bovine animals have greater amounts of β2 cellular receptor than β1 (Mersmann, 1998); thus, ZH can provide greater effect on muscle hypertrophy compared with RH in beef cattle supplementation. Similar to the present study, Frese et al. (2016) reported higher CK concentrations in animals fed ZH than in those fed RH and control diets and stated that it could be associated with many factors, including myocardial infarction, damage of skeletal muscle, and increase in lean body mass. The same authors proved that diets containing β-AA have no effect on arrhythmia rates, which suggests that an increase in CK concentration represents an increase in lean body mass. In the present study, creatinine and CK concentrations were positively correlated with slaughter weight, *Longissimus* muscle area, hot carcass weight, dressing, and total weight of retail cuts, which also may suggest an increase in muscle deposition.

5. Conclusions

Combining immunocastration with β-adrenergic agonists supplementation modifies lipid metabolism without affecting protein metabolism. Immunocastration enhances lipogenesis and reduces skeletal muscle accretion.

Feeding zilpaterol hydrochloride reduces protein catabolism. Moreover, blood metabolites related to lipolysis remain unchanged, which may suggest that zilpaterol hydrochloride has a smaller effect on adipose tissue lipolysis compared with skeletal muscle accretion.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: D.S. Antonelo and S.L. Silva. Formal analysis: D.S. Antonelo and N.R.B. Cônsolo. Funding acquisition: S.L. Silva. Investigation: D.S. Antonelo and S.L. Silva. Methodology: D.S. Antonelo, J.F.M. Gómez, M.R. Mazon, K.E.Z. Nubiato and S.L. Silva. Project administration: D.S. Antonelo, M.R.

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