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Ruminants

Performance and carcass characteristics of goats fed crude glycerin in the feedlot and during pre-slaughter lairage

Carolina Pilar Alves e Dias[1], Fredson Vieira e Silva[1*], Aylle Medeiros Matos[1], Laura Lúcia dos Santos Oliveira[1], Amilton Maia Freitas de Oliveira[1], Vicente Ribeiro Rocha Júnior[1], Leandro Farias Batista[1], Valéria Dias Martins[1]

[1] *Universidade Estadual de Montes Claros, Departamento de Ciências Agrárias, Janaúba, MG, Brasil.*

ABSTRACT - This study evaluated the effects of crude glycerin (CG) supplied separately from the other dietary ingredients to goats during the feedlot and in the pre-slaughter lairage on the nutrient intake, performance, behavioral and physiological parameters, carcass characteristics, and meat quality. Twenty-eight uncastrated male goats from a cross between the Boer breed and mixed-breed goats were used in a completely randomized design in a 2 × 2 factorial scheme, consisting of the feedlot with or without CG inclusion in the diets (96.90 g kg^{-1} DM) and the pre-slaughter lairage, also with or without the ingredient. In the feedlot, goats fed CG had a lower intake of dry matter and nutrients. Crude glycerin intake reduced the weight gain and body weight of goats and increased feed conversion. Weights of carcass and cuts (leg, loin, ribs, and shoulder) were lower when the animals consumed CG in the feedlot. In pre-slaughter lairage, concentrations of cortisol were lower when the animals consumed CG. Characteristics of carcass and meat (pH, color, water holding capacity, cooking loss, and shear force) were not changed by the supply of CG during the pre-slaughter. When supplied during the feedlot period and separately from the other dietary ingredients, CG negatively affected the performance and carcass characteristics of goats without changing meat quality. In the pre-slaughter lairage, CG intake lowers the cortisol level and does not change the behavioral parameters or carcass and meat characteristics of goats.

Key Words: carcass quality, meat quality, slaughterhouse, welfare

Introduction

The incentive for biodiesel production as a source of renewable energy has led to the production of large amounts of crude glycerin (CG) (ANP, 2016). Crude glycerin can be used in animal feeding due to its high acceptability and energy value (Donkin et al., 2009; Parsons et al., 2009; Terré et al., 2011; Pellegrin et al., 2012). This compound has been used at different inclusion levels in ruminant feed (intervals of 25 to 550 g kg^{-1}). When supplied at 100 g kg^{-1} of the dry matter (DM) of diets for feedlot animals in the concentrate or in the water, it does not reduce nutrient intake, performance, or carcass and meat characteristics (Borges et al., 2013; Chanjula et al., 2014a; Silva et al., 2018). In addition to its use in the feeding of animals in feedlot, it is presumed that this feed can serve as a source of energy for pre-slaughter kids.

During the pre-slaughter handling, physiological stress and physical exhaustion may promote expenditure of glycogen, which is associated with meat quality (Del Campo, 2016). In the lairage period, Parker et al. (2007) and Egea et al. (2015) used glycerol as a strategy of prophylactic hyperhydration to reduce the energy deficit and for the maintenance of body water as an osmotic agent, preserving health and increasing glucose production.

As far as it is known, CG has not been studied separately from the dietary ingredients in the feedlot and in pre-slaughter lairage. The hypothesis tested in this study is that CG improves the performance and carcass and meat characteristics of goats. Moreover, when supplied in pre-slaughter lairage, it improves animal welfare and contributes to improve carcass and meat traits.

Material and Methods

The procedures adopted in this experiment were approved by the local Ethics Committee in Animal Experimentation and Welfare (CEEBEA) (case no. 093/2016). The experiment was performed in Janaúba, Minas Gerais, Brazil, located at 15º43'47" S latitude, 43º19'18" W longitude, and of 516 m altitude.

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***Corresponding author:** fredson.silva@unimontes.br

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The study involved 28 uncastrated male goats originating from a cross between Boer breed and mixed-breed animals, with an average initial weight of 16.96±1.66 kg and an average age of 90 days. Goats were distributed in a 2 × 2 factorial scheme in completely randomized design (place – feedlot or slaughterhouse; and food – water or CG) with seven replicates. In the feedlot, CG was supplied separately from the other ingredients. In the slaughterhouse, CG was provided during the 12 h that the animals remained in the stalls.

Animals were housed in individual 1.5-m^2 stalls equipped with feeders, drinkers, and wood shavings beds. The experimental period in the feedlot was 74 days, the first 18 days used for adaptation to the diets and facilities.

In the feedlot, two diets were formulated with a 40:60 roughage:concentrate ratio (Tables 1 and 2).

The crude glycerin, obtained from vegetable oil, was donated by the Petrobras Bicombustível S.A. industry (Montes Claros, MG, Brazil). The proportion of this ingredient in the diet was set at 96.90 g kg^{-1} of the total DM. This value was pre-fixed based on information from the scientific literature in which authors concluded that this rate did not change the intake or nutrient digestibility of goat and sheep diets (Gunn et al., 2010; Borges et al., 2013; Chanjula et al., 2014a; Silva et al., 2018). Diets were formulated to meet the requirements of crude protein and metabolizable energy and provide a gain of 200 g day^{-1} during the feedlot period, following the NRC (2007). Concentrate and forage were supplied twice a day, at 07:00 and 16:00 h. Crude glycerin was provided to the animals in a separate drinker, in its entirety, in the morning.

Table 1 - Compositions of the experimental diets (g kg^{-1} of dry matter)

	Control	Glycerin
Ingredient		
Tifton hay 85	391.10	420.50
Corn meal	374.60	221.30
Extruded soybean	203.30	230.60
Mineral salt[1]	24.70	24.70
Calcitic limestone	6.20	5.90
Crude glycerin	-	96.90
Chemical composition		
Dry matter	879.90	856.00
Crude protein	145.50	158.70
Ether extract	14.50	18.50
Neutral detergent fiber	411.50	343.20
Total carbohydrates	614.20	598.70
Non-fibrous carbohydrates	202.60	235.50
Glycerol		89.10

[1] Guaranteed levels per kg product: Ca, 30 g; P, 21 g; S, 31 g; Na, 78 g; Mg, 4 g; Zn, 600 mg; F, 210 mg; Mn, 200 mg; Fe, 300 mg; Se, 1.5 mg; Cu, 35 mg; Cr, 3.5 mg; Mo, 45 mg; I, 8 mg; Co, 1.8 mg.

Animals had water available *ad libitum*. Adjustments were made to the diet by weighing the orts of all feedstuffs, including CG; these orts were fixed at 10% of the total amount offered. Feed intake was determined by subtracting the daily orts from the total provided.

Samples of the diet of each animal were collected for later chemical analyses of DM, ash, ether extract, and crude protein contents of orts from forage and concentrate, following the methodology proposed by AOAC (1995). Neutral detergent fiber corrected for ash and crude protein was obtained by following the recommendations of Detmann et al. (2012). Total carbohydrates were estimated using the equation proposed by Sniffen et al. (1992). To estimate the non-fibrous carbohydrates, we employed the equation proposed by the NRC (2001). The crude protein, ash, ether extract, and DM contents in the CG were obtained by the above-described procedures in analyses of the feeds. Glycerol and methanol contents were obtained by gas chromatography with a FID CG 17A detector (Shimadzu, Kyoto, Japan).

Animals were weighed at the end of the adaptation period and at the end of the feedlot stage, after feed deprivation for 16 h. With these data, we determined their average daily weight gain and feed conversion.

In the feedlot, the animals were subjected to behavioral assessments for four 24-h periods by visual observation of their feeding behavior, which took place at 5-min intervals (Mezzalira et al., 2011). The following behavioral variables were studied: feeding (concentrate, roughage, or CG intakes), water intake, rumination, and idle (Azevedo et al., 2013).

At the end of the feedlot period, animals were deprived of solid diets for 16 h. Subsequently, they were transported to the slaughterhouse over a distance of 311 km. Goats were loaded into the vehicle, transported, and unloaded as recommended by Costa et al. (2008).

Table 2 - Analysis of the concentrate, Tifton 85 hay, and crude glycerin (g kg^{-1} of dry matter)

Composition	Control concentrate	Glycerin concentrate	Hay	Crude glycerin
Dry matter	926.90	926.50	807.30	707.50
Crude protein	215.83	270.80	102.40	1.40
Ether extract	30.96	27.42	18.60	1.37
Minerals	78.97	97.61	39.00	66.00
Neutral detergent fiber	136.23	135.89	598.90	0.00
Total carbohydrates	660.43	590.72	715.70	917.20
Non-fibrous carbohydrates	530.36	459.42	116.80	917.00
Glycerol	-	-	-	916.20
Methanol	-	-	-	5.00
Density (g mL^{-1})	-	-	-	1.23

In the slaughterhouse, as in the feedlot, animals were distributed individually into groups of animals that received CG and water and animals that received only water. The amount of CG provided per animal was 0.970 kg. Water and CG were available to the animals until immediately before slaughter. Animals were filmed during the pre-slaughter lairage to measure the time the goats spent lying (lateral or ventral recumbency), according to methodology proposed by Silva et al. (2017). Prior to slaughter, goats had their blood drawn by jugular venipuncture into Vacutainer® tubes (BD Diagnostics, São Paulo, Brazil). Three tubes were collected: one with anticoagulant (EDTA), one containing potassium fluoride, and another without anticoagulant. The mean corpuscular volume was calculated by the formula of Wintrobe (Birgel, 1982). Total protein, albumin, creatine kinase, urea, and glucose values were measured in a spectrophotometer, using commercial kits (Doles®). For analysis of hemoglobin and non-esterified fatty acids (NEFA), we used the Bioclin® kit and the Randox® kit, respectively. For the chemical analysis of cortisol, the chemiluminescence method was applied.

Slaughter commenced 12 h after the animals arrived at the slaughterhouse. Animals were stunned by electronarcosis, consisting of two electrodes placed on their head, which was immediately followed by bleeding in accordance with the Brazilian Federal Meat Inspection Regulations (Brasil, 2017).

Hot carcasses were weighed, washed, and taken to a cooling chamber with a temperature ranging from 2 to 4 ºC for 24 h. After cooling, carcasses were weighed again, and the ultimate pH was measured in the loin (*LM thoracics et lumborum*), as described by Pearce et al. (2010), using a portable pH meter. Carcass cuts were obtained in leg, loin, rib, and shoulder. A 10-cm section of deboned *longissimus* muscle was removed from the carcass after 24 h of cooling. Meat color, conductivity, water loss by cooking, water holding capacity, and shear force were assessed on this portion of the loin.

Lightness (L*), redness (a*), and yellowness (b*) values were measured according to the description of Devine et al. (2002) using the Hunter lab Miniscan model Miniscan EZ. The conductivity was measured using the Tec-3MP Tecnal instrument.

To determine water loss by cooking, meat was weighed and placed on an electric grill. Cooking was made with cuts of the loin wrapped in aluminum foil. When the temperature at the coldest point on the steaks reached 40 ºC, they were turned over, and the other side was grilled until reaching 71 ºC. Meat sections were then cooled to room temperature and weighed again (Ramos and Gomide, 2007).

Three cylindrical samples were taken from the meat sections that underwent cooking. Samples of 1.27 cm in diameter were used to perform the objective analysis of shear force, which was measured using a Warner-Bratzler type device (Wheeler et al., 2001).

The part of the raw longissimus muscle that remained after removing the steaks for cooking was used to evaluate the water holding capacity. These samples were subjected to centrifugation (4 min/1500 rpm) and placed in an oven (18 h/70 ºC). The water holding capacity was calculated by the difference in weight (Nakamura and Katoh, 1981).

When significant ($P<0.05$), the dependent variables related to performance, carcass characteristics, and meat quality traits were adjusted as a function of the co-variable initial body weight. For the physiological variables, the respective co-variables corresponded to the measurements performed at the start of the feedlot period. In the case of variables measured only in the feedlot, means were compared by the t test ($P<0.05$). For the variables lying, water intake, and crude glycerin intake, which were determined at the slaughterhouse, the assumptions of normality for the residuals and homogeneity of variance were not accepted. Therefore, the Kruskal-Wallis test was applied for the lying and water intake variables, while the Mann-Whitney was applied for crude glycerin intake ($P<0.05$). The physiological variables and the carcass and meat characteristics were analyzed using the procedure of model with fixed effects. In this model, the main effects were the feedlot, pre-slaughter lairage, and their interaction. After the analysis of variance, means were compared by the Student-Newman-Keuls (SNK) test, in case the interaction was significant ($P<0.05$).

The mathematical model used was:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha_i\beta_j + e_{ijk},$$

in which y_{ijk} = observed value of the variable that received CG in feedlot i, that received CG in slaughterhouse j, and repetition k; μ = overall mean; α_i = feedlot – control diet or diet with CG; β_j = slaughterhouse – water or CG; $\alpha_i\beta_j$ = interaction effect; and e_{ijk} = random error associated with each observation.

Results

The intakes of DM, crude protein, ether extract, mineral matter, neutral detergent indigestible fiber, organic matter, and total carbohydrates were lower for animals that ingested CG (Table 3). Goats that received CG also showed lower weight gain and body weight and same feed conversion than the animals in the control group. The time spent feeding, ruminating, ingesting water, and idling was similar between animals with or without CG inclusion in the diets. In pre-slaughter lairage, there was no influence of the CG availability on the time the goats spent lying (Table 4). Water and CG intakes were also not changed by the supply of CG.

There was no interaction between feedlot and pre-slaughter lairage on the studied physiological parameters of goats. In the feedlot, animals that consumed CG displayed lower albumin levels (Table 5); the other afore-mentioned variables remained similar. In pre-slaughter lairage, these variables were not different between animals that received CG and those that did not.

Cortisol concentration of kids did not differ in the feedlot. In pre-slaughter lairage, goats consuming CG presented lower levels for this variable. Glucose, total protein, urea, and NEFA concentrations of goats were similar.

In the feedlot, the carcass weights and yields of animals were lower for the group consuming CG (Table 6). The weights of the cuts (leg, ribs, and shoulder) were also higher in goats that did not receive the ingredient in the feedlot. The loin-eye area of goats showed no alteration by the supply of CG. In pre-slaughter lairage, in turn, no effect of CG inclusion was detected for the carcass characteristics and weights of cuts.

The ultimate pH values of carcasses of kids were similar in goats that received CG and those that did not. Lightness (Hunter L*) and conductivity of the meat from goats that consumed CG only in the feedlot was lower. The CG intake of goats in the feedlot and pre-slaughter lairage periods caused no changes in water-holding capacity, cooking loss, or shear force of meat.

Discussion

Goats preferred the solid portion of the diet over the CG, which directly influenced the DM intake. Unlike the present findings, Chanjula et al. (2014b) evaluated the inclusion of glycerin in goat diets at levels of up to 200 g kg^{-1}

Table 3 - Nutrient intake, performance, and ingestive behavior of goats finished with diets with or without crude glycerin

Item	Control	Glycerin	SE	P-value
Intake (g day^{-1})				
Dry matter	889.93	677.41	35.50	0.001
Crude protein	164.98	136.59	6.75	0.034
Ether extract	28.44	19.55	1.24	0.000
Neutral detergent fiber	412.39	265.66	20.42	0.000
Ash	47.84	37.34	1.90	0.004
Organic matter	842.09	640.07	33.60	0.001
Total carbohydrate	430.20	279.71	20.96	0.000
Non-fiber carbohydrates	538.65	359.34	23.98	0.000
Performance				
Average daily gain (g)	214.88	156.62	6.80	0.000
Feed conversion	4.18	4.45	0.19	0.474
Final body weight (kg)	29.92	26.87	0.68	0.005
Ingestive behavior (h day^{-1})				
Feeding	3.60	3.75	0.17	0.665
Ruminating	6.59	5.94	0.60	0.166
Water intake	0.18	0.14	0.03	0.269
Idle	13.62	14.17	0.37	0.316

SE - standard error.

Table 4 - Water intake (kg), crude glycerin intake (g), and lying behavior (min) of goats during pre-slaughter lairage

Item	Treatment				Percentile		P-value
	CG/CG	CG/W	W/CG	W/W	25th	75th	
Water	2.36	1.40	1.77	2.43	0.47	3.21	0.990
Glycerin	460.14	-	64.61	-	39.29	537.97	0.480
Lying behavior	497.00	574.00	457.00	509.00	423.00	623.00	0.160

Treatments (feedlot/pre-slaughter): CG/CG - glycerin available/glycerin available; CG/W - glycerin available/glycerin unavailable; W/CG - glycerin unavailable/glycerin available; W/W - glycerin unavailable/glycerin unavailable.

Table 5 - Physiological parameters of goats fed crude glycerin during feedlot and pre-slaughter lairage

Item	Feedlot (F)		Pre-slaughter (P)		SE	P-value		
	Glycerin	Control	Glycerin	Control		F	P	F × P
MCV (fL)	38.27	36.84	36.22	38.86	2.55	0.701	0.478	0.688
Glucose (mg dL^{-1})	78.92	79.85	78.01	80.76	1.98	0.702	0.149	0.087
Urea (mg dL^{-1})	72.89	76.83	71.09	78.63	3.37	0.412	0.140	0.053
Albumin (g L^{-1})	24.40	26.70	25.70	25.30	0.07	0.037	0.827	0.330
Total protein (g L^{-1})	69.20	69.50	67.30	71.40	0.17	1.000	0.090	0.985
NEFA (mmol L^{-1})	5.10	6.30	5.30	6.10	0.04	0.073	0.223	0.510
Creatine kinase (mg dL^{-1})	0.51	0.54	0.57	0.49	35.29	0.533	0.301	0.285
Cortisol (ng mL^{-1})	7.50	6.40	5.50	8.40	0.06	0.344	0.003	0.320

MCV - mean corpuscular volume; NEFA - non-esterified fatty acids; SE - standard error.

Table 6 - Carcass and meat characteristics of goats fed crude glycerin during feedlot and pre-slaughter lairage

Item	Feedlot (F)		Pre-slaughter (P)		SE	P-value		
	Glycerin	Control	Glycerin	Control		F	P	F × P
HCW (kg)	10.47	12.51	11.59	11.39	0.38	0.000	0.679	0.500
HCY (%)	39.01	41.74	40.73	40.01	0.53	0.000	0.290	0.332
Ultimate pH[1]	5.57a	5.79a	5.63a	5.56a	0.06	0.243	0.282	0.030
CCW (kg)	9.89	11.88	10.90	10.88	0.36	0.000	0.940	0.623
CCY (%)	36.84	39.67	38.29	38.22	0.64	0.003	0.938	0.700
LEA (cm^2/kg CCW)	1.06	1.00	1.07	0.99	0.04	0.310	0.209	0.356
Leg (kg)	1.33	1.58	1.46	1.45	0.05	0.001	0.824	0.686
Loin (kg)	0.55	0.67	0.60	0.62	0.04	0.050	0.627	0.704
Rib (kg)	1.71	2.04	1.89	1.87	0.06	0.001	0.809	0.634
Shoulder (kg)	1.12	1.36	1.25	1.24	0.04	0.000	0.850	0.203
Lightness (L*)[1]	29.65a	23.83b	26.16ab	28.51a	1.07	0.645	0.210	0.010
Redness (a*)	3.67	3.98	3.86	3.79	0.22	0.377	0.834	0.275
Yellowness (b*)[1]	6.45a	5.59a	5.63a	6.42a	0.37	0.958	0.878	0.031
Conductivity (mV)[1]	71.53ab	57.28b	65.17ab	75.95a	3.97	0.175	0.667	0.009
Shear force (kg)	4.64	5.60	5.11	5.13	0.35	0.059	0.967	0.168
CWL (g 100 g^{-1})	40.62	37.84	39.81	38.65	1.44	0.276	0.642	0.339
WHC (g 100 g^{-1})	20.63	19.39	19.03	20.97	1.04	0.429	0.221	0.087

HCW - hot carcass weight; HCY - hot carcass yield; CCW - cold carcass weight; CCY - cold carcass yield; LEA - loin-eye area; CWL - cooking weight loss; WHC - water holding capacity; SE - standard error.
[1] Treatments (feedlot/slaughterhouse): glycerin/glycerin, glycerin/water, water/glycerin, and control (water/water), respectively.
Means in the same line with different letters are different (P<0.05).

DM and reported no alterations in animal growth-related parameters.

The decreased DM intake in the diet with CG led to a lower nutrient intake. At the same time, worse results for weight gain and body weight were found in this study, without interfering with feed conversion. Gomes et al. (2011) found no differences in DM intake, feed conversion, daily weight gain, or body weight with glycerin inclusion (300 g kg^{-1} DM) in the diet for lambs. Unlike the present work, the afore-mentioned authors mixed CG with other diet ingredients, which was the possible reason for the different results. The greater acceptability of soybean and corn would possibly increase CG intake of goats, if these ingredients were mixed.

The similar results for the feeding behavior-related variables are likely associated with the similar chemical composition of the diets, which were calculated to be isoenergetic and isonitrogenous and to have the same proportion of neutral detergent fiber (Table 1). According to Carvalho et al. (2011), the chemical composition of the feed, especially in terms of neutral detergent fiber contents, can directly influence the feeding behavior of ruminants, since this component is the main factor interfering with the rumen functioning.

Contrasting with this experiment, in relation to water and CG intakes, Pethick et al. (2000) reported that sheep fed a mixture of glycerol and propylene glycol had their water intake doubled for a period of one to two days.

Concentrations of albumin, total proteins, urea, and mean corpuscular volume are used for the evaluation of the hydration status (Thrall et al., 2015). Although albumin concentrations indicate a higher water intake for animals that consumed CG, no differences were found in the water-intake behavior (Table 3). According to Parker et al. (2007), the prophylactic treatment with glycerol in sheep results in over-hydration.

Cortisol is commonly used to evaluate experimental situations involving animal welfare, as indicator of fear and excitement (Broom and Fraser, 2010). Stress stimulates the hypothalamic-adrenal cortex axis with subsequent release of cortisol (Zimerman et al., 2013, Chulayo et al., 2016). Cortisol in turn stimulates gluconeogenesis from amino acids and glycerol and elevates the blood NEFA concentrations (Lehninger, 2006). High concentrations of cortisol, in association with hunger due to long periods of fasting in pre-slaughter, lead to an increase in protein catabolism (Kannan et al., 2000; Zimerman et al., 2013). Therefore, the greater stimulation of gluconeogenesis in the treatment without CG might have resulted in similar glucose concentrations between the treatments. However, no greater mobilization of glucogenic amino acids or triglycerides was verified in the treatment without CG because the total protein, urea, and NEFA concentrations were similar between the treatments. Because there was no interaction between feedlot and pre-slaughter lairage, the benefit from the use of CG in relation to the cortisol concentration in pre-slaughter does not depend on the previous supply of this ingredient in the feedlot.

Since the performance of animals that consumed CG in feedlot was lower (Table 3), the carcass characteristics and weights of the cuts also had quantitative losses, as also reported by Lage et al. (2014). Observing the result for loin-eye area, it was noted that the amount of edible portion of the carcasses remained similar between the treatments. Carvalho et al. (2015) also found that carcass weight decreased as CG was included in the diet (up to 30 g 100 g^{-1} DM) and that loin-eye area was not altered.

For Aberle et al. (2001), the ultimate pH in the muscle of animals not subjected to starvation or exercise prior to slaughter is below 5.7. Because the performance obtained with the treatments including the ingredient was inferior (Table 3), one can hypothesize that CG intake in lairage mitigates the problem of animals consuming it in the feedlot, given that lightness was higher in the other treatments. Conductivity followed the same pattern as lightness. Conductivity and lightness had an inverse relationship with the final pH (Lawrie, 2005, Velarde et al., 2000), which makes this result coherent. Likewise, Borghi et al. (2016) found no changes in water holding capacity, cooking losses, and shear force in meat of lambs fed diets containing different levels of crude glycerin. Similarly, Lage et al. (2014) observed no changes in ultimate pH, cooking losses, and shear force in meat of lambs fed diets containing different levels of crude glycerin. The average values for shear force in all treatments of this study were considered by Souza et al. (2004) as the upper limit for considering the meat as tender. Overall, the meat quality traits of the goats studied here were similar to those found in other studies with crossbred goats and were within the range associated with good quality (Beserra et al., 2001; Borges et al., 2006; Monte et al., 2007).

Conclusions

Crude glycerin provided separately from the other dietary ingredients to goats in the feedlot period negatively affects their performance and carcass characteristics, without changing the meat quality. In pre-slaughter lairage, crude glycerin reduces the cortisol levels, but does not alter behavior parameters or carcass and meat characteristics.

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