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Testosterone, Cortisol and Weight Gain in Confined Texel Lambs Supplemented with Commercial Probiotic and its Association with Selenium and Chromium

Testosterona, Cortisol e Ganho de Peso em Cordeiros Texel Confinados Suplementados com Probiótico Comercial e sua Associação com Selênio e Cromo

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Abstract

The aim of this study was to evaluate lambs' supplementation with the yeast Saccharomyces cerevisiae, with or without added selenium and chromium, on the plasma levels of testosterone, cortisol and weight gain. Twenty-four male Texel lambs were used, mean age of 120 days and weight 29.0 ± 2.44 kg, divided into 3 groups: G-Control (commercial feed without additives), G-Pro (commercial feed + 5 g yeast Saccharomyces cerevisiae), and G-Pro+Se/Cr (commercial feed + 3 g yeast Saccharomyces cerevisiae + 50 mg/kg organic selenium + 300 mg/kg organic chromium). Weight measurement and blood sampling were performed for testosterone and cortisol measurement in the 6th, 9th, and 18th weeks after the start of supplementation. There was an increase ($p<0.05$) in testosterone levels in the 9th week in the G-Pro (2.02 ± 0.83 ng/mL) compared to the G-Control (0.99 ± 0.84 ng/mL). Weight gain did not differ ($p=0.5117$) between groups. We concluded that the use of probiotics increases testosterone, but the addition of microelements did not change testosterone and both products did not impact weight gain or cortisol concentration.
Keywords: Testosterone. Cortisol. Yeast. Sheep. Saccharomyces cerevisiae.

Resumo

O objetivo do estudo foi avaliar a suplementação de cordeiros com a levedura Saccharomyces cerevisiae, com ou sem adição de selênio e cromo, sobre os níveis plasmáticos de testosterona, cortisol e ganho de peso. Foram utilizados 24 cordeiros machos da raça Texel, com idade média de 120 dias e peso de 29,0 ± 2,44 kg. Divididos em 3 grupos: G-Controle (ração comercial sem aditivos), G-Pro (ração comercial + 5 g de levedura Saccharomyces cerevisiae) e G-Pro+Se/Cr (ração comercial + 3 g de levedura Saccharomyces cerevisiae + 50 mg/kg de selênio orgânico + 300 mg/kg de cromo orgânico). Foram realizadas pesagem e coleta de sangue para dosagem de testosterona e cortisol na 6ª, 9ª e 18ª semanas após o início da suplementação. Houve aumento ($p<0,05$) nos níveis de testosterona na 9ª semana no G-Pro (2,02 ± 0,83 ng/mL) em comparação ao G-Controle (0,99 ± 0,84 ng/mL). O ganho de peso não diferiu ($p=0,5117$) entre os grupos. O uso de probióticos aumenta a testosterona, mas a adição de microelementos não altera a testosterona e ambos os produtos não impactaram o ganho de peso ou a concentração de cortisol.
Palavras-chave: *Testosterona. Cortisol. Levedura. Ovinos. Saccharomyces cerevisiae.*

1 Introduction

The increase in world's population requires maximization of animal production to ensure sustainable sources of meat, milk and other products of animal origin (Evans et al., 2022). To this end, biologically active substances can be added to the diet to increase productivity at lower cost. In the last decade, the use of antibiotics in animal production has been reduced and probiotics have been included in feed (Abd El-Tawab et al., 2016). In contrast to antibiotics (which have a direct bactericidal or bacteriostatic effect on bacteria), probiotics act via indirect mechanisms, such as changes in the gut microbiome, increased gut efficiency and modulation of the host's innate immune response (Buntyn et al., 2016), in addition to stabilizing rumen pH and increasing volatile fatty acid production (Jia et al., 2018).

Yeasts targeting a better uptake of microelements have been used together with organic selenium (Se) in production systems to improve production performance and carcass quality (García et al., 2015). Selenium is an essential trace element for humans and animals, which is required in sufficient quantities for bone metabolism, immunity, the endocrine system, iodine metabolism and reproduction (Muegge; Brennan, 2016) and has an important antioxidant function (Mehdi; Dufrasne, 2016).

However, soil selenium concentrations are low in many parts of the world, including the state of São Paulo, where the study is to be conducted (Gabos; Alleoni; Abreu, 2014). Animals fed with roughage grown on selenium-deficient soils and not supplemented with minerals become more susceptible to stress, leading to clinical consequences, including reproductive problems and low resistance to infectious diseases (Van Metre; Callan, 2001).

Chromium (Cr) used in supplementation of animals reduces plasma cortisol (Lashkari; Habibian; Jensen, 2018).

Several strategies have been investigated to reduce the stress to which animals are exposed during routine handling on a farm, including the effects of chromium supplementation in ruminant diets to reduce serum cortisol levels (Morgado et al., 2023; Glombowsky et al., 2024).

In this study, we hypothesized that the use of probiotics in finishing lambs would promote an increase in weight gain, while the association of probiotics with microelements such as selenium and chromium would enhance this effect and increase testosterone concentrations. The aim was therefore to investigate the effects of supplementing lambs with Saccharomyces cerevisiae, which is contained in commercially available products with (Yea-Sacc®) or without (Beef-Sacc®) selenium and chromium, on weight gain and plasma testosterone concentration.

2 Material and Methods

2.1 Local

The experiment was performed from April to August 2016, at a property located in the city of Presidente Prudente, São Paulo state, Brazil. The geographical coordinates of the property are latitude 22º 07' S and longitude 51º 27' W, with an altitude of 430 m. The climate is characterized as humid subtropical (McKnight; Hess, 2000). The average amounts of annual rainfall and in the months of April, May, June, July, and August were 4.3 mm, 5.1 mm, 1.1 mm, 0.6 mm, 4.0 mm, respectively. The average annual temperature in the city of Presidente Prudente was 24.4 ºC (12.3 ºC to 31.1 ºC) and the minimum and maximum temperatures during the experimental period were 12.3 ºC to 30.3 ºC. (Meteorological Station of UNOESTE, 2016).

The experiment was carried out in accordance with the guidelines of the Ethics Committee on the usage of animals in experiments and was approved by the scientific committee (CEA/UNOESTE 3461).

2.2 Animals

Twenty-four pure breed Texel male lambs from the same origin were used, aging 120 days, with an average weight of 29.0 ± 2.44 kg, which were subjected to 21 days diet adaptation period. The animals were kept in the fold throughout the experimental period (2m²/lamb, concrete floor and sawdust bedding).

2.3 Groups and Feeding

The animals were distributed in three experimental groups through a completely randomized design and all groups received the same basic diet, consisting of 80% commercial concentrate and 20% Coast-cross hay. The chemical composition of the diet is described in Table 1.

Table 1 - Bromatological composition of Coast-cross hay and commercial feed®, supplied to Texel lambs (n = 24) in confinement

Bromatological composition (%)	Coast-cross hay	Commercial feed®
Crude Protein	8.32	18.00
Crude Fiber	34.09	19.00
Mineral Matter	3.87	-
Ethereal extract	1.57	4.60
Total Digestible Nutrients	55.50	74.00

Source: research data.

The test groups consisted of: Control group (G-Control, n = 8) fed with the basal diet only; Probiotic group (G-Pro, n = 8) fed with the basal diet plus yeast consisting of 5 x 109 CFU/g Saccharomyces cerevisiae strain 1026 (Yea-Sacc®, Alltech, Nicholasville, EEA), with animals receiving 5.0 g of probiotic/animal/day, according to the manufacturer›s recommendation for the respective species; and probiotic/microelement group (G-Pro Se/Cr, n = 8) fed with 5 x 106 CFU/g Saccharomyces cerevisiae strain 1026 in combination with 50 mg/kg selenium and 300 mg/kg organic chromium (Beef Sacc®, Alltech, Nicholasville, EEA), totaling 3.0 g of probiotic/animal/day, according to the manufacturer›s recommendation for the respective species.

Mineral salt was made available ad libitum, containing the following composition: guaranteed levels per kg of product: phosphorus (min) 65 g/kg, calcium (min) 120.00 g/kg, calcium (max) 160.00 g/kg, sodium (min) 147.00 g/kg, magnesium (min) 10.00 g/kg, flavoring 10.00 g/kg, cobalt (min) 40.00 mg/kg, iron (min) 1,800 mg/kg, fluorine (max) 870.00 mg/kg, iodine (min) 75.00 mg/kg, manganese (min) 1,300.00 mg/kg, selenium (min) 25.00 mg/kg, zinc (min) 4,160.00 mg/kg.

The basal feed was calculated according to the recommendations of the NRC (2006) to cover the nutritional requirements of these animals with a weight gain of 250 g/day and adjusted according to the lambs' development.

2.4 Hormonal dosing and weighing

Three blood samples were taken 6, 9 and 18 weeks after the start of the supplementation. The samples were collected by jugular vein puncture (Vacutainer®), then centrifuged at 1500 g/10 min and the blood plasma was stored at -20 °C for further processing with the radioimmunoassay method (RIA). Commercial kits (MP Biomedicals, LLC., Solon, OH) for the measurement of testosterone were used. The intra-assay coefficient of variation for testosterone was 5.51% for the high control and 0.15% for the low control, with a detection sensitivity of 0.46 ng/mL.

The lambs were weighed individually, always in the morning and before the first feeding, to determine body weight and weight gain.

2.5 Statistical analysis

The data were subjected to an analysis of variance

(ANOVA), and in the case of a significant difference, the mean values were compared using the Tukey test at a 5% level of significance ($p<0.05$).

3 Results and Discussion

The plasma testosterone concentrations, body weight and weight gain are shown in Table 2. The testosterone concentration in G-Pro was higher than in G-Control in the 9th week of supplementation and did not differ from G-Pro + Se/Cr. Body weight and weight gain did not differ between the groups at the three time points evaluated.

Table 2 - Mean values ± standard deviation of plasma concentrations of testosterone, body weight and weight gain between periods, evaluated in Texel breed lambs (n = 24) with 6, 9 and 18 weeks of supplementation

Testosterone (ng/mL)	G-Control (n = 8)	G-Pro (n = 8)	G-Pro+Se/Cr (n = 8)	p-value
6th week	1.05 ± 0.62	0.99 ± 0.89	0.71 ± 0.58	0.6365
9th week	0.99 ± 0.84 b	2.02 ± 0.83 a	1.08 ± 0.70 ab	0.0450
18th week	2.75 ± 2.01	3.57 ± 2.10	2.59 ± 1.95	0.6497
Body weight (Kg)				
6th week	34.93 ± 3.60	34.86 ± 4.35	34.56 ± 3.72	0.9814
9th week	37.66 ± 3.66	38.29 ± 4.90	37.43 ± 3.72	0.9110
18th week	55.01 ± 3.41	56.01 ± 3.56	54.06 ± 3.21	0.5321
Weight gain (kg)				
6th week	2.74 ± 1.01	3.43 ± 1.17	2.86 ± 0.60	0.3298
9th week	17.35 ± 2.54	17.73 ± 2.15	16.61 ± 2.99	0.7023
18th week	20.09 ± 3.05	21.15 ± 1.97	19.50 ± 3.03	0.5117

* Different letters on the same line differ from each other by the Tukey test ($P<0.05$). G-Control - fed only with the basal diet, G-Pro (basal diet + 5g of Probiotic Saccharomyces cerevisiae), G-Pro+Se/Cr (basal diet + 3g of Probiotic Saccharomyces cerevisiae associated with 50 mg/kg of selenium and 300 mg/kg of organic chromium).
Source: research data.

In the last decade, the use of nutritious antibiotics has been banned totally or partially in many countries, and as an alternative, probiotics are widely used as feed additives in livestock animals and have been defined as non-pathogenic microorganisms. (Abd El-Tawab et al., 2016). In ruminant nutrition, the most commonly used probiotic microorganism is Saccharomyces cerevisiae (Syngai et al., 2016)pigs and poultry, and also in aquaculture practices. In this review, the microorganisms frequently used as probiotics in human and animal welfare has been described, and also highlighted are the necessary criteria required to be fulfilled for their use in humans on the one hand and on the other as microbial feed additives in animal husbandry. Further elaborated in this article are the sources from where probiotics can be derived, the possible mechanisms by which they act, and their future potential role as antioxidants is also discussed. (Syngai et al., 2016. Although previous studies report the potential of probiotics in the manipulation of the gastrointestinal microbiota and consequently in weight gain, their effectiveness generally varies between studies and results are inconsistent (Jia et al., 2018; Burdick Sanchez et al., 2020).

In the current study, we supplemented lambs for 126 days (18 weeks) with probiotic or probiotic plus selenium and chromium, and the testosterone levels were higher in the group receiving only probiotics. In the 9th week of supplementation (approximately 180 days of life), an increase in testosterone was observed in the G-Pro group, but it was unique and did not occur at other assessment times, so we believe it is related to the onset of early puberty in some animals in this group. An increase in testosterone concentration was seen just before puberty, after the age of 7 months (Henrique et al., 2020). The mean testosterone levels of the 6 months in this study were similar to the value of 2.73±0.68 ng/mL observed by Henrique et al. (2020) for Santa Ines lambs.

In another study with goats, the incorporation of selenium in the supplementation with probiotic induced an increase in blood perfusion in the supratesticular artery, with an increase in FSH, LH, IGF1 and testosterone (Mandour et al., 2020) the influence of supra-nutritional organic selenium (Se. This increase in testosterone caused by the use of probiotic with selenium is due to actions in increasing LH (Mojapelo; Lehloenya, 2019; Mandour et al., 2020)spermatozoa abnormalities and acrosome damage compared to the control. Supplementation with selenium significantly ($P < 0.05$ or a direct effect of selenium on testicular interstitial cells through its antioxidant properties, or by increasing testicular irrigation (Mandour et al., 2020)the influence of supra-nutritional organic selenium (Se.

In relation to weight gain, it can be observed that the animals' weight over the experimental period showed positive linear growth. However, supplementation with probiotic or probiotic plus selenium and chromium did not alter the animals' weight gain. Arcos-García et al. (2000) when evaluating the effect of two microbial cultures containing Saccharomyces cerevisiae, in the dosages of 1g/day and 3g/day, used in the direct feeding of Suffolk sheep (30kg/LW), did not find improvements in the animal performance. On the other hand, Haddad and Goussous (2005), evaluating the effect of adding 0, 3, and 6 grams per day of the yeast Saccharomyces cerevisiae in 24 lambs of the Awassi breed

(20.6 ± 0.2 kg/LW) during finishing, observed an increase in animal's performance with the dosage of 3g/day. Although numerous studies have reported significant benefits from the use of probiotics (regardless of species), in others the results have indicated little or no effect (Buntyn et al., 2016).

Chromium is a mineral that acts in the metabolism of glucose, from insulin and some studies have shown benefits of chromium supplementation for ruminants (Griss et al., 2020)the effects of supplementation with organic chromium and yucca extract (alone or in combination, improving energy transport, as well as immunity (Da Rocha et al., 2013). However, in the current study, we did not observe an effect of adding this microelement to the probiotic.

We hypothesized in this study that the use of probiotic with the addition of selenium and chromium in confined lambs would promote greater weight gain, both through the direct action of the probiotic in the rumen environment, and by chromium and selenium, increasing the antioxidant response and increasing an plasma testosterone concentration. However, our hypothesis was not proven and based on the results obtained, it appears that the observed alterations were due to the higher dose of probiotic (5 g) and not to the incorporation of selenium or chromium to the 3 g dose of Saccharomyces cerevisiae.

4 Conclusion

The use of the commercial probiotic or its association to selenium and chromium do not alter the weight gain of lambs in feedlot, even with a transitory increase in testosterone in the probiotic group and no effect in the cortisol concentration.

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