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## **ARTICLE IN PRESS**

# Effects of yeast and exogenous fibrolytic enzyme inclusion in the diet of hair lambs on performance, carcass traits, physicochemical parameters and meat fatty acid profile

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(Saccharomyces cerevisiae, Yeast) were supplemented individually or in combination in lamb diets to investigate their impact on performance, and physicochemical, lipid, and sensory attributes of meat. Forty male lambs were assigned to five treatments in a completely randomised design [control diet, control plus yeast, control plus EFE, blends of 0.7Yeast + 0.3EFE and 0.7EFE + 0.3Yeast (g/kg dry matter)]. The diets did not impact performance and carcass traits. Meat from lambs fed control diets and the 0.7Yeast + 0.3EFE mixtures exhibited lower shear force (P = 0.03). The meat redness value and saturation index (C\*) were lower in lambs fed yeast compared to the control diet, while lightness was more intense in meat from lambs fed the 0.7Yeast + 0.3EFE blend in comparison to the other treatments (P < 0.05). Meat from lambs fed the 0.7Yeast + 0.3EFE mixture showed higher concentrations of C12:0 (P = 0.048) and C14:0 (P = 0.01) saturated fatty acids, and obtained higher scores on a hedonic scale for tenderness and succulence (P < 0.01). The addition of the 0.7Yeast + 0.3EFE blend, or Yeast and EFE separately, to the diet resulted in a higher concentration of C18:2 n-6 (P < 0.01) in the Longissimus lumborum (LL) muscle. Additionally, the inclusion of additives led to increased concentrations of C20:4 polyunsaturated fatty acids (PUFA) (P = 0.046) in LL compared to the control diets. The incorporation of the 0.7Yeast + 0.3EFE mixture improved tenderness and colouration of meat, as evaluated by consumers, and increased PUFA levels in LL.

ABSTRACT. An exogenous fibrolytic enzyme (EFE) and a yeast strain

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## Introduction

Yeast and exogenous fibrolytic enzymes (EFE) are feed additives used in ruminant diets to improve

rumen fermentation and fibre digestibility. They are able to enhance rumen fermentation efficiency by stimulating the growth of Gram-negative anaerobic bacteria, which play a fundamental role in the processes occurring in this environment. The inclusion of yeast and EFE in the ruminant diet is associated with enhanced fermentation and microbial protein synthesis, thereby improving meat yield, carcass finishing, tenderness, and nutritional properties (Amin and Mao, 2021).

Dietary supplementation with yeast (Saccharomyces cerevisiae) can improve water retention and meat tenderness due to increased activity of proteolytic enzymes in muscles and reduced oxidative stress, which helps preserve meat quality (Sowińska et al., 2016). Obeidat (2017) supplemented S. cerevisiae in the diet of growing Awassi lambs and found no significant benefits on the growth performance of the animals tested. On the other hand, Moharrery and Asadi (2009) observed an increase in daily weight gain when finishing sheep were supplemented with a malate-yeast mixture. These discrepancies in results may be attributed to factors such as the type and level of yeast in the diet, the health status of the animals, the experimental period, or the species of animals evaluated (Torres et al., 2022).

Supplementing EFEs can also enhance animal performance by improving fibre digestion and increasing the total hydrolytic capacity of the rumen, ultimately leading to more efficient feed utilisation and faster weight gain (Song et al., 2018). EFEs play a crucial role in breaking down complex plant cell wall materials, such as cellulose and haemicellulose. When added to the diet, these enzymes can improve the digestibility of fibrous feeds by increasing the availability of nutrients to sheep (Zhou et al., 2023). This, in turn, supports better rumen fermentation and production of volatile fatty acids (VFA). Additionally, improved fibre digestion can reduce gut fill, enabling sheep to consume more nutrients without overloading their digestive system (Clauss and Hummel, 2017). Overall, the combined application of yeast and fibrolytic enzymes can enhance the nutritional value of sheep diets, promote efficient digestion of fibrous materials, increase VFA production, and contribute to improved meat quality in terms of growth rate, meat tenderness, and flavour (Salami et al., 2019). These nutritional strategies are valuable tools in optimising sheep production systems for better overall performance and meat quality. Furthermore, the addition of EFEs can increase meat tenderness, as well as protein and essential amino acid contents (Zhou et al., 2023). Tirado-González et al. (2021) applied EFE dietary supplementation and observed alterations in the fat composition of meat, notably, reduced saturated fatty acids (SFA) and increased polyunsaturated fatty acids (PUFA) contents. Nevertheless, the administration of yeast and EFE as feed additives for ruminant animals is contingent on factors such as the dosage, diet composition, and method of incorporation, necessitating consideration of these aspects to achieve the goal of producing higher-quality meat (Amin and Mao, 2021).

We therefore hypothesised that the addition of yeast in combination with EFE to the diet of lambs may exert synergistic effects on dietary fibre digestibility, and consequently on rumen microbial activity, resulting in greater weight gains and improved meat quality components. The objective of this study was to investigate the effects of the inclusion of yeast (*S. cer-evisiae*) and EFE individually or in a mixture in different proportions on the quality, fatty acid profile and sensory characteristics of meat from confined lambs.

## Material and methods

## Ethical considerations, animals, experimental treatments, handling, diets and chemical composition

The trial was conducted at the experimental farm of the School of Veterinary Medicine and Animal Science of the Federal University of Bahia (UFBA), in São Gonçalo dos Campos – Bahia, Brazil. The research was previously approved by the Ethics Committee on the Use of Animals (CEUA) at the School of Veterinary Medicine and Animal Science of UFBA, under protocol CEUA/UFBA 14/2015.

The treatments included a control diet and four diets with additives applied separately or in combinations as follows (Figure 1): 1. control group – standard diet without additives; 2. Yeast – 100% exclusive addition of yeast at a level of 1 g/kg diet

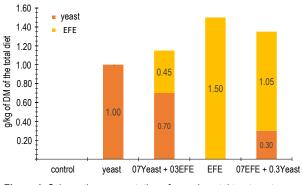


Figure 1. Schematic representation of experimental treatments

Yeast – Saccharomyces cerevisiae, EFE – exogenous fibrolytic enzyme, DM – dry matter; diets: control – no additive, Yeast – 1.0 g/kg DM, 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet

dry matter (DM), representing 100% of the level recommended by the manufacturer; 3. 0.7 Yeast + 0.3 EFE - inclusion of 70% of yeast (0.7 g/kg total diet DM) and 30% EFE (0.45 g/kg total diet DM); 4. EFE - 100% inclusion of exogenous fibrolytic enzyme at a level of 1.5 g/kg total diet DM, representing 100% of the level recommended by the manufacturer; and 5. 0.7EFE + 0.3Yeast - inclusion of 70% of exogenous fibrolytic enzyme (1.05 g/kg total diet DM) and 30% of yeast (0.3 g/kg total diet DM). The additives were mixed with the concentrate at the time of feeding, adhering to the reference levels recommended by the manufacturer: 1.5 g/kg DM of EFE (Fibrozyme; composed of the enzyme xylanase; Alltech do Brasil Agroindustrial Ltda, Maringa – Paraná, Brazil) and 1.0 g/kg DM of yeast (Yea-Sacc; Saccharomyces cerevisiae; Alltech do Brasil Agroindustrial Ltda, Maringa – Paraná, Brazil).

Forty uncastrated Santa Ines male lambs, with an average age of five months and an initial body weight of  $25.0 \pm 1.3$  kg, were selected, dewormed and distributed in a completely randomised design. Animals were housed individually in 1.0 m<sup>2</sup> stalls with suspended slatted wooden floors and equipped with feeders and drinkers. The experimental phase lasted 96 days, including an initial 15-day adjustment period for acclimatisation to the environment, handling, and diet introduction.

All animals were fed twice daily at 08:00 and 16:00. The amount of feed provided was calculated to allow for up to 200 g/day of leftovers, and water was provided ad libitum. The roughage to concentrate ratio was set at 40:60, with Tifton-85 hay (Cvnodon sp.) constituting the forage (400 g/kg DM), ground into approximately 5.0 cm particles; the concentrate (600 g/kg DM) was composed of ground maize (425 g/kg DM), soybean meal (160 g/ kg DM) and mineral mixture (15 g/kg DM), the latter having the following composition per kg of product: 120 g of calcium, 87 g of phosphorus, 147 g of sodium, 18 g of sulphur, 590 mg of copper, 40 mg of cobalt, 20 mg of chromium, 1800 mg of iron, 80 mg of iodine, 1300 mg of manganese, 15 mg of selenium, 3800 mg of zinc, 300 mg of molybdenum, a maximum of 870 mg of fluoride. The diets were formulated according to NRC (2007) guidelines for a weight gain of 200 g/day.

The chemical composition of the ingredients and diets is shown in Table 1. The contents of DM (method 967.03), mineral matter (MM, method 942.05), ether extract (EE, method 920.29), and crude protein (CP, method 981.10) were determined according to the methods outlined by AOAC International (2015).  
 Table 1. Chemical and fatty acid composition of ingredients and experimental diets

Variables	Ground maize	Soybean meal	Tifton-85 hay	Diet
Composition, % DM				
DM, g/kg as fed	86.4	86.9	86.6	86.8
crude ash	1.16	6.68	7.49	6.06
crude protein	8.9	49.18	5.55	13.9
ether extract	2.14	0.96	0.84	1.40
apneutral detergent fibre§	8.36	14.4	76.9	36.6
acid detergent fibre§	1.97	7.88	37.4	17.1
acid detergent fibre	0.12	0.55	7.85	3.28
non-fibre carbohydrates	79.4	28.8	9.25	42.1
total digestible nutrients	82.3	80.2	55.0	69.8
Fatty acid composition, g/100 saturated fatty acids (SFA)	-			
C12:0	2.96	0.80	3.30	2.71
C14:0	1.08	0.00	1.76	1.23
C14.0	1.03	5.33	1.49	1.23
C16:0	21.8	12.3	13.5	16.9
C17:0	1.86	5.8	3.06	2.94
C18:0	5.41	7.48	1.61	4.14
monounsaturated fatty acids		1.10		
C14:1	7.36	2.33	10.6	7.74
C15:1	5.66	1.18	6.10	5.03
C16:1	0.75	5.26	3.25	2.46
C17:1	0.96	3.13	3.88	3.33
C18:1 cis	9.43	11.7	6.88	8.63
polyunsaturated fatty acids (F	PUFA)			
C18:2 cis	23.4	33.9	17.8	22.5
C18:3 n–6	6.95	0.61	1.17	3.52
C18:3 n–6	1.04	5.79	20.1	9.81
C20:2	1.78	0.33	0.84	1.15
C20:3 n–6	2.87	0.55	0.94	1.68
C20:3 n–6	1.78	1.43	1.58	1.62
C20:4	2.60	1.18	1.42	1.86
C20:5	1.21	0.56	0.71	0.89
∑SFA	34.2	32.1	24.7	29.9
∑MUFA	24.2	23.6	30.7	27.2
∑PUFA	41.7	45.3	44.1	42.9

DM – dry matter, FAME – fatty acid methyl ester; <sup>§</sup>corrected for ash and protein; C12:0 – lauric acid, C14:0 – mristic acid, C15:0 – pentadecanoic acid, C16:0 – palmitic acid, C17:0 – marginal acid, C18:0 – stearic acid, C14:1 – myristoleic acid, C15:1 – pentadecanoic acid, C18:1 – palmitoleic acid, C17:1 – heptadecanoic acid, C18:1 *cis* – oleic acid, C18:2 *cis* – linoleic acid, C18:3 n-6 – y-linoleic acid, C18:3 n-6 – a-linoleic acid, C20:2 – eicosadienic acid, C20:3 n-6 – eicosatrienoic acid cis 8, 11, 14, C20:3 n-6 – eicosatrienoic acid cis 11, 14, 17, C20:4 – lignoceric acid, C20:5 – eicosapentaenoic acid

The content of neutral detergent fibre (NDF) and acid detergent fibre (ADF) was determined according to the methodology described by Van Soest et al. (1991), with modifications for nonwoven tissue (Senger et al., 2008) and the use of thermostable amylase to remove starch. The NDF content was corrected for ash and protein ( $_{ap}$ NDF), according to the formula of Licitra et al. (1996). The acid detergent lignin (ADL) content was determined using method 973.18 (AOAC International, 2015), involving the action of 72% sulphuric acid on the ADF residue. Non-fibre carbohydrates (NFC) were calculated according to the equation described by Weiss (1999): *NFC* (%) = 100 - (%<sub>ap</sub>NDF + %*CP* + %*EE* + %ash). The total digestible nutrient (TDN) content was derived from equations estimating the digestibility of the analytical fractions.

# Slaughter, carcass traits and commercial meat cuts

Daily DM intake was calculated based on the diet offered and orts collected to determine the chemical composition of the diets. The chemical composition analysis involved creating pooled samples of the diets, ingredients and leftovers. At the end of the experimental period, animals were weighed after a 16-h period of constant fasting to obtain body weight at slaughter (BWS). Following BWS determination, animals underwent electronarcosis, following the guidelines of the Federal Inspection Service (SIF) for humane slaughter as per Normative nº03/00, MAPA regulations (Brazil, 2000). Subsequently, bleeding (by cutting the jugular veins and carotid arteries), skinning, and evisceration was carried out, followed by inspection of the viscera by specialised technicians. After removing the organs, legs, head and skin, the carcasses were weighed to determine hot carcass weight (HCW), and then stored for 24 h in a cold chamber (4 °C) before being weighed again to obtain cold carcass weight (CCW).

The pH was measured directly after slaughter and 24 h later, between the  $12^{th}$  and  $13^{th}$  rib, using a digital potentiometer with a skewer-type tip directly on the *longissimus lumborum* muscle. Before analysis, the digital potentiometer was calibrated using buffer solutions of pH 4 and 7 at controlled temperature. Three measurements were taken for each animal, and the average value of these three measurements was used as the representative pH value. Subsequently, left and right *longissimus lumborum* muscles were dissected, packaged, labelled and stored in a freezer (-20 °C) for further evaluation of physicochemical composition (excluding colour analysis) and fatty acid (FA) profile.

#### **Physicochemical meat parameters**

Before freezing, a fresh sample of the *longis-simus lumborum* muscle was cut out, allowing it to equilibrate at temperatures between 6 and 7 °C for 40 min for colour assessment (Biffin et al., 2019).

A Minolta CR-400 colorimeter (Konica Minolta, Tokyo, Japan) was calibrated before each analysis using a white tile standard. After 30 min of exposure to the atmosphere for myoglobin oxygenation, measurements were conducted in triplicate using the CIE system (Commission Internationale de l'Éclairage) for the following indices: L\* – luminosity (L\* 0 = black; 100 = white), a\* – redness, and b\* – yellowness (Miltenburg et al., 1992). The saturation index (chroma, C\*) was determined from the a\* and b\* data, according to the equation: C\* =  $[(a^*)^2 + (b^*)^2]^{0.5}$  (Boccard et al., 1981).

To determine water holding capacity (WRC) of the *longissimus lumborum* muscle, samples of approximately 5.0 g were collected, placed between circular Albert 238 paper filters (12.5 cm diameter) and subjected to a 10 kg load for 5 min (Hamm, 1986). The samples were subsequently weighed, and the WRC was calculated from the difference in the weight of the samples before and after the load placement.

The determination of cooking loss (CL) was conducted following the recommendations of the American Meat Science Association (AMSA, 2015). Assessments were performed in duplicate on 2.5-cm thick samples without subcutaneous fat. The meat was pre-weighed and cooked until the geometric centre reached 71 °C on a grill (George Foreman<sup>®</sup> Jumbo Grill GBZ6BW, Rio de Janeiro, Brazil) using a stainless-steel thermocouple (Gulterm 700; Gulton do Brasil). After cooking, the steaks were cooled to room temperature for stabilisation, and then weighed to calculate CL from the difference in the weight of the samples before and after cooking, with values expressed as % of exudate released.

To evaluate Warner-Bratzler shear force (WBSF), three central samples, approximately 1.0 cm in diameter and 2.0 cm in length, were collected from the steaks, parallel to the muscle fibres. The shear force was measured using a Texture Analyzer TX-TX2 (Mecmesin, Nevada, USA), equipped with a Warner-Bratzler shear blade, applying a load of 25 kgf and a cutting speed of 20 cm/min. The shear force values obtained were expressed in Newtons (N) according to the standard procedure recommended by the Meat Animal Research Center (Shackelford et al., 1999). Moreover, the analyses of meat chemical composition (moisture, protein, fat and ash) were performed using FoodScan<sup>™</sup> (France) infrared ray scanning.

#### Meat fatty acid profile

The FA profile determination was carried out on the basis of FA methyl esters (FAME) in samples of dietary ingredients (Table 1) and lyophilised samples of the *longissimus lumborum* muscle. The samples were processed according to the method described by O'Fallon et al. (2007), utilising a solution of potassium hydroxide, methanol, sulphuric acid, hexane and an internal standard (C19:0).

The FA composition was determined by a gas chromatography using a Supelco<sup>®</sup> Analytical SPTM-2560 capillary column, 100 m  $\times$  0.25 mm  $\times$  0.20 µm (Supelco® InC., Bellefonte, PA, USA), installed in a Focus GC Thermo Scientific gas chromatograph (Thermo Electron SpA®, Milan, Italy). The initial oven temperature was 140 °C, subsequently increasing to 220 °C at a rate of 1 °C/min and maintained for 25 min. Hydrogen was used as the carrier gas at a flow rate of 1.5 ml/min. The injector temperature was maintained at 250 °C and the detector at 280 °C. The injection volume was 1 µl and the split ratio was 30:1. Fatty acids were identified by comparing retention times with chromatographic reference standards (Nu-Chek Prep, Inc.), and their concentrations in the *longissimus lumborum* muscle were expressed as mg/100 g meat, and in ingredients relative to total FAME (as g/100 g FAME).

The sums of saturated fatty acids ( $\sum$ SFA), monounsaturated fatty acids ( $\sum$ MUFA), polyunsaturated fatty acids ( $\sum$ PUFA), and omega 3 and 6 fatty acids (n-3 and n-6, respectively), as well as the  $\sum$ SFA: $\sum$ PUFA and  $\sum$ n-6: $\sum$ n-3 ratios, were calculated from the identified fatty acid profiles of each sample.

The total sums of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), as well as the  $\Sigma PUFA:\Sigma SFA$  and  $\Sigma n-6:\Sigma n-3$  ratios, were calculated based on the determined fatty acid profiles. To assess the nutritional quality of the lipid fraction of the longissimus lumborum muscle, the atherogenicity index (AI) was calculated using the following equation: AI =  $[(C12:0 + (4 \times C14:0) + C16:0)]/$  $(\Sigma MUFA + \Sigma n-6 + \Sigma n-3)$  and TI = (C14:0 + C16:0)+ C18:0)/[( $0.5 \times \Sigma$ MUFA) + ( $0.5 \times \Sigma$ n-6 + ( $3 \times$  $\Sigma$ n-3) + ( $\Sigma$ n-3/ $\Sigma$ n-6)] (Ulbricht and Southgate, 1991); the hypocholesterolaemic to hypercholesterolaemic fatty acid ratio (h:H ratio) was calculated using the following formula: h:H = (C18:1 cis-9 +C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + C20:5 n-3)/ (C14:0 + C16:0) according to Santos-Silva et al. (2002). Desired fatty acids (DFA) were determined according to the formula described by Rhee (1992), where DFA = (MUFA + PUFA + C18:0).

The enzymatic activities of  $\Delta$ 9-desaturase C16 ( $\Delta$ 9C16),  $\Delta$ 9-desaturase C18 ( $\Delta$ 9C18), and elongase were estimated following the equations of Smet et al. (2004):  $\Delta$ 9C16 = [C16:1/(C16:0 + C16:1)] × 100,  $\Delta$ 9C18 = [(C18:1cis-9)/(C18:0 + C18:1cis-9)]

 $\times$  100 and elongase = [(C18:0 + C18:1cis-9)/(C16:0 + C16:1 + C18:1cis-9)]  $\times$  100.

#### Sensory analysis

A panel of 80 untrained consumers (Stone and Sidel, 1985) was employed to assess the sensory attributes of lamb meat. Lamb samples, derived from the longissimus lumborum muscle of the right loin, were coded and prepared. The preparation involved baking in an electric oven preheated to 170 °C and monitored with continuous monitoring using a digital spit thermometer (Incoterm®, Bahia, Brazil) until the internal temperature reached 71 °C. Subsequently, the baked samples were cut into cubes of approximately 1.0 cm<sup>3</sup>. To ensure minimal loss of heat and aroma volatiles, the samples were coded and kept in a water bath (Thermomix<sup>®</sup>, São Paulo, Brazil) in plastic jars, covered with aluminium foil and lids at 75 °C to maintain the desired temperature range of 65–70 °C during the sensory test.

For the sensory evaluation, each participant received one sample of each treatment in plastic containers with lids, uniquely coded with three random digits. The samples were served on disposable plates, and cream crackers were offered between tests to neutralise any potential residual flavours.

The sensory panel was conducted between 09:00 and 11:00 in individual booths. The evaluation of sensory attributes was carried out using a nine-point scoring scale. Tasters evaluated the following sensory attributes: flavour, tenderness, juiciness, 'goat' flavour and smell (Madruga et al., 2015), general acceptability, and preference. The possible scores, ranging from 1 to 9, were as follows: 1 – disliked extremely; 2 – disliked very much; 3 – disliked moderately; 4 – disliked slightly; 5 – indifferent; 6 – liked slightly; 7 – liked moderately; 8 – liked very much; and 9 – liked extremely.

#### Statistical analysis

The experimental design was a completely randomised approach with five treatments and eight experimental units (lamb/treatment), with the initial weight of the animals used as a covariate to reduce the random error of initial weight. The data were analysed using the MIXED procedure of SAS<sup>®</sup> 9.3. In the analysis, the treatment was considered a fixed effect, and the sampling error as a random effect according to the following model:  $Y_{ijk} = \mu + \tau_i + \varepsilon_{ijk}$ , where:  $Y_{ijk}$ = value k observed in the experimental unit that received treatment i, replication j;  $\mu$  = overall average common to all observations;  $\tau_i$  = effect of treatment i;  $\varepsilon_{ijk}$  = random error with mean 0 and variance.

For sensory analysis, a nine-point hedonic scale was employed wherein participants selected a response from nine alternatives for each feature under evaluation. The data exhibited a polynomial distribution, allowing for the application of a class of models based on the exponential family. Consequently, the generalised linear models (GLM) approach was applied, taking advantage of the multinomial nature of the data. Unlike methods involving mathematical transformations for data normalisation, the GLM approach incorporates the inherent data distribution characteristics. The transformation occurs solely on the systematic component of the model, thereby enhancing the statistical power of the test. Analysis of deviance (ANODEV), a generalisation of ANOVA for GLM, was performed using the SAS® 9.1 GEN-MOD procedure (SAS Institute, 2003.).

All data were compared using the Tukey test for multiple comparisons. The effects were deemed significant at  $P \le 0.05$ .

## Results

No significant effects were observed on DMI, final weight, hot and cold carcass weight and yield, subcutaneous fat thickness and loin eye area of lambs from the groups administered dietary additives (Table 2). The yield of commercial meat cuts: neck, shoulder, shank, loin, and rib was not affected by the addition of yeast and EFE separately or in combinations.

However, the additives in the lamb diets affected the shear force (P = 0.03) of the *longissimus* lumborum muscle (Table 3). Lambs that were fed the control diet presented greater Warner-Bratzler shear force (WBSF) compared to those administered the 0.7Yeast + 0.3EFE blend and lower WBSF compared to those given the EFE alone. Meat redness (a\*; P = 0.02) and chroma value (C\*; P = 0.02) were lower in lambs fed diets with yeast, EFE, and 0.7EFE+0.3 yeast compared to the control diet, while lightness (L\*; P = 0.038) was more pronounced in the meat from lambs provided the 0.7Yeast + 0.3EFE diet compared to the other treatment groups. No differences were found in the meat of lambs fed the additive-enriched diets in terms of initial and final pH, cooking loss, water holding capacity, b\* colour indices, moisture, protein, fat, and ash content.

As shown in Table 4, meat from lambs fed the 0.7Yeast + 0.3EFE diet contained higher concentrations (g/100 g FAME) of C12:0 (P = 0.048) and C14:0 (P = 0.009) SFA. The administration of the 0.7Yeast + 0.3EFE diet or diets with yeast and EFE supplemented individually resulted in higher levels of C18:2 n-6 (P = 0.003) in *longissimus lumborum*, as well as higher concentrations of C20:4 (P = 0.046) PUFA in the meat compared to the control animals. MUFA (Table 5), other saturated fatty acids, PUFA, the sums and relationships between SFA, as well as nutraceutical indices in lamb meat were not affected (P > 0.05) by the inclusion of yeast and EFE as dietary additives.

 Table 2. Growth performance, carcass attributes and commercial meat cuts of lambs fed diets containing yeast (Saccharomyces cerevisiae) and/or exogenous fibrolytic enzyme (EFE)

				Diets				
Variables	control	yeast	0.7Yeast + 0.3EFE	EFE	0.7EFE + 0.3Yeast	SEM	P-value*	
Performance								
dry matter intake, kg/day	1.10	1.20	1.22	1.05	1.15	54.3	0.20	
initial weight, kg	25.4	23.8	25.0	25.8	26.4	0.34	0.66	
final weight, kg	40.13	39.95	40.60	42.13	42.85	0.73	0.681	
Carcass traits								
hot carcass weight, kg	17.73	18.58	18.83	17.08	18.53	0.34	0.518	
cold carcass weight, kg	17.68	18.55	18.75	17.0	18.40	0.34	0.643	
hot carcass yield, %	44.8	44.4	44.6	43.1	44.6	0.28	0.681	
subcutaneous fat thickness, mm	2.63	2.79	2.63	2.63	3.08	0.10	0.527	
loin eye area, cm <sup>2</sup>	16.24	15.08	16.96	15.0	15.24	0.36	0.336	
Meat cuts yield, %								
neck	20.50	19.47	19.76	19.50	19.95	0.13	0.688	
shoulder	18.34	19.03	19.87	19.27	19.05	0.09	0.233	
shank	29.38	28.87	29.19	29.69	29.09	0.11	0.168	
loin	14.24	13.16	12.54	12.88	13.08	1.18	0.083	
rib	17.54	19.47	18.42	18.68	18.83	0.10	0.096	

Diets: control – no additive, yeast – 1.0 g/kg dry matter (DM), EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet; SEM – standard error of the mean; 'significance at P < 0.05 according to Tukey's test

				Diets			
Variables	control	yeast	0.7Yeast + 0.3EFE	EFE	0.7EFE + 0.3Yeast	SEM	<i>P</i> -value <sup>*</sup>
Initial pH	7.13	7.14	7.11	7.19	7.15	0.02	0.761
Final pH	6.03	6.05	5.98	6.10	6.13	0.02	0.489
Cooking loss, %	11.0	9.25	12.3	10.2	11.9	0.50	0.884
Water holding capacity, %	70.0	70.6	69.6	70.6	71.0	2.40	0.635
Walter Bazer Shear Force, N	20.4 <sup>b</sup>	20.6 <sup>b</sup>	18.0°	24.3ª	20.5 <sup>b</sup>	0.14	0.033
Colour indices							
L* (lightness)	39.00 <sup>ab</sup>	38.30 <sup>b</sup>	41.25ª	37.93⁵	38.77 <sup>b</sup>	0.34	0.038
a* (redness)	22.1ª	20.9 <sup>b</sup>	21.9ª	21.0 <sup>⊳</sup>	21.3⁵	0.15	0.024
b* (yellowness)	5.99	5.10	5.74	4.93	5.60	1.04	0.133
C* (chroma)	22.9ª	21.5⁵	22.6ª	21.6 <sup>⊳</sup>	<b>22</b> .1⁵	0.18	0.018
Composition, %							
moisture	71.5	70.7	71.6	71.9	71.5	0.27	0.783
protein	19.9	19.7	19.9	19.8	19.7	0.11	0.627
fat	2.35	2.15	2.29	2.08	2.46	0.35	0.603
ash	6.25	7.45	6.21	6.22	6.34	0.08	0.540

Table 3. Meat quality of lambs fed diets containing yeast (Saccharomyces cerevisiae) and/or exogenous fibrolytic enzyme (EFE)

Diets: control – no additive, yeast – 1.0 g/kg dry matter (DM), EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet; SEM – standard error of the mean; 'significance at P < 0.05 according to Tukey's test, <sup>abc</sup> – indicates that there are differences between the means

Table 4. Individual fatty acid profile (g/100 g fatty acids methyl ester (FAME)) of *longissimus lumborum* of lambs fed diets containing yeast (Saccharomyces cerevisiae) and/or exogenous fibrolytic enzyme (EFE)

<b>F</b> _44 +				Diet			
Fatty acids, g/100 g FAME	control	yeast	0.7Yeast + 0.3EFE	EFE	0.7EFE + 0.3Yeast	SEM	<i>P</i> -value <sup>*</sup>
Saturated fatty acids							
C12:0	0.17°	0.79ª	0.32 <sup>b</sup>	0.29 <sup>b</sup>	0.14	0.09	0.048
C14:0	2.99 <sup>b</sup>	4.99ª	3.36 <sup>ab</sup>	3.15⁵	2.67	0.33	0.009
C16:0	24.5	24.2	24.3	24.1	24.6	0.45	0.773
C18:0	15.6	15.5	13.3	16.0	15.2	0.78	0.951
Monounsaturated fatty acids	;						
C16:1 <i>cis</i> 9	2.52	2.73	2.76	2.21	2.43	0.08	0.975
C18:1 <i>cis</i> 9	44.1ª	40.1 <sup>b</sup>	44.8ª	38.9 <sup>₅</sup>	45.4	1.13	0.021
Polyunsaturated fatty acids							
C18:2 n-6	1.64°	2.37ª	2.04ª	2.10 <sup>ab</sup>	1.95⁵	0.14	0.003
<sup>1</sup> CLA (C18:2 c9t11)	0.32	0.32	0.30	0.28	0.29	0.01	0.547
C18:3 n-3	0.13	0.13	0.11	0.16	0.14	0.06	0.826
C20:4 n-6	0.44 <sup>b</sup>	0.70ª	0.60ª	0.62ª	0.63ª	0.05	0.046
C20:5 n-3	0.06	0.06	0.05	0.05	0.06	0.01	0.905

Diets: control – no additive, yeast – 1.0 g/kg dry matter (DM), EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet; CLA – conjugated linoleic acid (rumenic acid + other isomers); C12:0 – lauric acid, C14:0 – myristic acid, C16:0 – palmitic acid, C18:0 – stearic acid, C16:1 *cis* 9 – palmitoleic acid, C18:1 *cis* 9 – oleic acid, C18:2 *cis* – linoleic acid, C18:3 n-3 – α-linoleic acid, C20:4 n-6 – arachidonic acid, C20:5 n-3 – eicosapentanoic acid cis 5, 8, 11, 14, 17; SEM – standard error of the mean; 'significance at P < 0.05 according to Tukey's test, <sup>ab</sup> – indicates that there are differences between the means

Meat from lambs fed the 0.7Yeast + 0.3EFE diet received higher scores on the hedonic scale for tenderness (P = 0.005) and succulence (P = 0.001) (Table 6). On the other hand, there was no visible effect (P > 0.05) of dietary supplements on the sensory attributes (flavour, goat flavour and odour, and over-

all acceptance) of the lamb meat, as perceived by the tasters. The scores on the hedonic scale assigned for flavour, tenderness, succulence and overall acceptance ranged from 6 (liked slightly) to 7 (liked moderately), while for goat flavour and odour they varied from 4 (slightly disliked) to 5 (indifferent).

Table 5. Sums, ratios, indices and enzymes (g/100 g fatty acids methyl ester (FAME)) from the *longissimus lumborum* muscle of lambs fed diets containing yeast (*Saccharomyces cerevisiae*) and/or exogenous fibrolytic enzyme (EFE)

Verieblee				Diets			
Variables, g/100 g FAME	control	yeast	0.7Yeast +0.3EFE	EFE	0.7EFE +0.3Yeast	SEM	P-value*
ΣMUFA	51.4	48.2	52.8	49.8	52.1	1.18	0.541
∑SFA	45.8	47.9	43.8	46.7	44.5	1.15	0.714
∑PUFA	2.82	3.91	3.38	3.53	3.37	0.22	0.219
∑PUFA:∑SFA	0.06	0.08	0.08	0.07	0.08	0.01	0.226
∑n-3	0.49	0.77	0.66	0.69	0.69	0.02	0.765
∑n-6	0.22	0.23	0.20	0.21	0.24	0.06	0.172
∑n-6:∑n-3	2.46	3.15	3.17	2.98	3.28	0.20	0.217
Atherogenicity index (AI)	0.70	0.94	0.73	0.88	0.68	0.05	0.366
Thrombogenicity index (TI)	1.59	1.78	1.52	1.65	1.56	0.58	0.579
h:H index	1.70	1.50	1.75	1.66	1.78	0.06	0.616
Desired fatty acids (DFA)	69.8	67.7	69.5	69.3	70.2	2.86	0.853
∆9-dessaturase C16	9.34	10.1	10.14	9.19	8.98	0.32	0.980
∆9-dessaturase C18	73.9	71.9	76.8	72.5	74.8	1.47	0.749
Elongase	68.8	67.3	68.1	68.6	69.10	0.44	0.431

Diets: control – no additive, yeast – 1.0 g/kg dry matter (DM), EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet; SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids, h:H – hypocholesterolaemic / hypercholesterolaemic ratio; SEM – standard error of the mean; 'significance at P < 0.05 according to Tukey's test

Table 6. Sensory attributes of the longissimus lumborum muscle of lambs fed diets containing yeast (Saccharomyces cerevisiae) and/or exog-	
enous fibrolytic enzyme (EFE)	

Attributes Control	Yeast	0.7Yeast +0.3EFE	EFE	0.7EFE +0.3Yeast	SEM	P-value*	
Flavour	6.84	6.46	6.60	6.60	6.86	0.20	0.30
Tenderness	7.33⁵	7.05 <sup>b</sup>	7.73ª	7.10 <sup>⊳</sup>	7.65ª	0.17	0.005
Succulence	6.69ª	6.33 <sup>b</sup>	6.28 <sup>b</sup>	6.60ª	6.93ª	0.17	0.001
Goat flavour	5.28	5.34	5.21	5.31	5.30	0.23	0.95
Goat odour	4.53	4.43	4.31	4.65	4.35	0.28	0.79
Overall acceptance	7.01	6.67	6.61	6.83	6.96	0.19	0.19

Diets: control – no additive, Yeast – 1.0 g/kg dry matter (DM), EFE – 1.5 g/kg DM EFE (Alltech<sup>®</sup>; Yea-Sacc e Fibrozyme – composed of xylanase enzyme), 0.7Yeast + 0.3EFE – 0.7g/kg yeast DM + 0.45 g/kg EFE DM of total diet, 0.7EFE + 0.3Yeast – 1.05 g/kg EFE DM + 0.3 g/kg yeast DM of total diet; Hedonic Scale ranging from 1 to 9 as follows: 1 – disliked very much, 2 – very displeased, 3 – disliked moderately, 4 – slightly disliked, 5 – indifferent, 6 – liked slightly, 7 – liked moderately, 8 – liked a lot, 9 – liked very much; SEM – standard error of the mean; 'significance at P < 0.05 according to Tukey's test, <sup>ab</sup> – indicates that there are differences between the means

## Discussion

Performance data and carcass traits remained unaffected by the inclusion of additives, and consequently, most parameters related to meat quality also did not show any significant changes in the present study. The lack of impact on these variables could be attributed to the fact that the basal diet was the same across all treatments, with the levels of additives possibly being insufficient to induce a substantial effect, particularly on fibre digestion and subsequently on DM intake (DMI). In addition, the diets contained high amounts of NDF, which resulted in similar weight gain and carcass characteristics for all the lambs. This contrasts with findings in the literature, where levels exceeding 4.0 g/day per animal led to divergent outcomes (Cagle et al., 2020). Issakowicz et al. (2013) analysed the effect of dietary supplementation of finishing lambs with yeast (*Saccharomyces cerevisiae*) as an additive and observed that yeast improved ADG and the DMI/ADG ratio; however, these authors no effects were observed on carcass characteristics and yields of commercial meat cuts in animals receiving highenergy diets (increased concentrate proportions). The mean values observed for SFT (2 and 5 mm), as well as for LEA (10 to 16 cm<sup>2</sup>), were also within the limits considered adequate for sheep, indicating a satisfactory effect of finishing diets on carcass characteristics (Silva Sobrinho, 2005). Similar results were obtained for the weight and yield variables of commercial meat cuts, which were also not affected by the inclusion of dietary additives. As there was no effect of yeast and EFE supplementation on total weight gain, consequently no differences were recorded in animal muscle development and the proportions of commercial meat cuts.

Meat colour and tenderness are extremely important indicators of lamb sensory qualities and consumer acceptability. The pH level plays a pivotal role, with higher values leading to darker meat, potentially influencing consumer purchasing decisions. In general, myoglobin pigments are responsible for redness a\*, L\* and b\* values, reflecting the water-holding capacity of raw meat (Prache et al., 2022). The colour of meat is collectively determined by the amount of myoglobin and haemoglobin and the level of lipid oxidation in muscle tissue. Some reports pointed out that the oxidation of myoglobin to metmyoglobin could cause a decrease in the a\* value (Fernández-López et al. 2005). The addition of yeast in the current study reduced the a\* and C\* colour indices and WBSF of meat, while increasing the L\* index, without altering the water content. This suggests that supplemental probiotics, such as yeast, may contribute to improved muscle colour and tenderness. Comparable results were observed in a study by Nie et al. (2022), where lambs fed yeast showed reduced shear force and C\* colouration, leading to an overall improvement in meat quality (Lawrie, 1985).

The additives also did not affect cooking loss and waterholding capacity values. However, the 0.7Yeats + 0.3EFE mixture promoted greater tenderness of lamb meat. The average WBSF values of lamb meat were lower than those reported in the literature (Bezerra et al., 2020). This could be related to the high pH values of the meat in the present study. It is plausible that animals receiving the additives presented greater tenderness in relation to meat of the control animals, as yeast are able to increase the activity of proteolytic enzymes, mainly calpains and cathepsins. These enzymes play a crucial role in breaking down muscle proteins during meat processing (Shackelford et al., 1999). There is also a possibility that the additives contributed to the reduction of oxidative stress in muscle cells (Nie et al., 2022). However, no discernible impact of additives on this variable was noted. The mean values of WBSF were below the threshold considered acceptable, i.e., up to 27 N force (Webb et al., 2005), indicating that meat tenderness in the present study could be considered adequate. The better meat tenderness observed in lambs fed the blends (0.7Yeast + 0.3EFE and 0.7EFE + 0.3Yeast) was further corroborated in the sensory attribute validation. Panellists classified the meat of lambs from this treatment as more tender and succulent, assigning higher scores on the hedonic scale (Hughes et al., 2014). The addition of yeast and EFE to the diets did not exert any significant effect on meat chemical composition parameters, which could be attributed to the uniformity of the diets across all treatments, with similar DM, fibre, and fat contents.

The analysis of the meat fatty acid profile revealed significant differences when yeast was applied as the sole additive. Yeast supplementation altered fat composition in meat, with higher contents of C12:0, C14:0 SFA and C18:2 n-6 and C20:4 n-6 PUFA. This observation is grounded in the potential of additives to influence rumen fermentation, leading to alterations in the fatty acid composition of meat. Altered in relationships between microbial populations in the rumen could result in modifications in SCFA production and consequently increase the synthesis of omega-3 and omega-6 fats in meat (Milewski and Zaleska, 2011). Moreover, the additives could increase fat absorption in the small intestine, thereby increasing their concentration in the bloodstream and in meat (Amin and Mao, 2021). However, despite these changes in fatty acid composition induced by additives in the lamb diet, they were not sufficient to alter the overall FA sums and ratios, nor did they affect enzymes and parameters associated with human health. Milewski and Zaleska (2011) observed an increase in PUFA concentrations without changes in sensory properties of lamb meat over a 100-day period when supplemented animals' diets with S. cerevisiae. However, in our study, we observed that the highest scores attributed for tenderness and succulence by the evaluators are correlated with the highest concentration of C20:4 n-6 PUFA in the meat of lambs fed with 0.7 Yeast + 0.3 EFE in the diet.

## Conclusions

It is recommended to incorporate *Saccharomyces cerevisiae* either individually or in combination with exogenous fibrolytic enzymes (EFE) at the levels tested in the present study (1.0 g/kg dry matter (DM) yeast and 1.5 g/kg DM EFE). This addition improves the tenderness of lamb meat, as indicated by higher scores given by panellists on the hedonic scale. Furthermore, this supplementation increased the concentrations of polyunsaturated fatty acids, which is particularly beneficial for enhancing meat lipid quality.

## **Conflict of interest**

The Authors declare that there is no conflict of interest.

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