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Performance and carcass characteristics of lambs fed diets supplemented with different levels of *Saccharomyces cerevisiae*

Adrian Gloria-Trujillo[1], David Hernández-Sánchez[1]*, María Magdalena Crosby-Galván[1], Omar Hernández-Mendo[1], Miguel Ángel Mata-Espinosa[2], René Pinto-Ruiz[3], Marco Antonio Ayala-Monter[4], Amada Isabel Osorio-Teran[5]

[1] Colegio de Postgraduados, Posgrado en Recursos Genéticos y Productividad – Ganadería, Texcoco, Estado de México, México.
[2] Universidad Autónoma Chapingo, Unidad Regional Universitaria de Zonas Áridas, Bermejillo, Durango, México.
[3] Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Chiapas, México.
[4] Universidad Autónoma de Guerrero, Escuela de Medicina Veterinaria y Zootécnia, Cuajinicuilapa, Guerrero, México.
[5] Universidad Autónoma del Estado de México, Centro Universitario Amecameca, Amecameca, Estado de México, México.

***Corresponding author:**
sanchezd@colpos.mx

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ABSTRACT - The objective of this study was to evaluate the productive performance, apparent digestibility, and carcass and *longissimus dorsi* muscle characteristics of lambs fed diets supplemented with four levels of *Saccharomyces cerevisiae*. Thirty-two male Hampshire lambs (25.82±1.95 kg body weight) were distributed in four treatments: basal diet (20:80, forage:concentrate), and the inclusion of 0, 3, 5, and 10 g animal^{-1} d^{-1} *Saccharomyces cerevisiae*. The variables evaluated were dry matter intake, daily weight gain, feed conversion, apparent digestibility, dorsal fat thickness, *longissimus dorsi* muscle area, and physicochemical characteristics of carcass and muscle. We used a completely randomized design and orthogonal polynomials to test the linear and quadratic effects of the inclusion levels of the yeast. *Saccharomyces cerevisiae* showed a quadratic effect on lamb performance. Dry matter intake decreased with yeast in response to a better feed conversion and body weight gain; however, at the highest *Saccharomyces cerevisiae* dose, daily weight gain and final weight were lower than with the basal diet treatment. *Saccharomyces cerevisiae* did not affect apparent digestibility or carcass and muscle physicochemical characteristics. Supplementation with 3 and 5 g d^{-1} *Saccharomyces cerevisiae* improves productive performance of lambs fed high concentrate diets without affecting the physicochemical characteristics of the carcass or muscle.

Keywords: *longissimus dorsi*, muscle characteristics, sheep, yeast

1. Introduction

The livestock sector is a dynamic, evolving system due to the growing demand for meat and milk, driven by increased incomes, growing population, and urbanization (Mottet et al., 2017). The production of lamb meat in the world is about 9 million t, and its demand increases each year (Mazinani and

Rude, 2020), making it necessary to design nutritional strategies that can improve production systems. In sheep, production systems range from extensive conditions to highly technified intensive regimes (Partida de la Peña et al., 2013). In intensive systems, the use of diets high in rapidly fermenting carbohydrates is common. These diets improve intrinsic and extrinsic carcass characteristics (Sañudo et al., 2007) through deposition of intramuscular and cover fat (Oliveira et al., 2017). But they also lead to increases in the production of volatile fatty acids and lactate, which decrease ruminal pH, undermine cellulolytic bacterial activity, and reduce fiber digestibility and production of microbial mass, as well as the productive capacity of lambs (Issakowicz et al., 2013).

For this reason, the use of feed supplements that improve animal digestive and productive efficiency has increased (Yirga, 2015), reflected in higher meat quality and yield (Vohra et al., 2016). In this sense, *Saccharomyces cerevisiae* is a probiotic capable of optimizing fiber digestion by eliminating oxygen dissolved in the rumen to improve lactate metabolism (Chaucheyras-Durand et al., 2008). In addition, *Saccharomyces cerevisiae* creates an optimal environment for development of fibrolytic bacterial species (Fonty and Chaucheyras-Durand, 2006), which affect dry matter intake and digestibility coefficients and result in improvement in animal yield (Fadel Elseed and Abusamra, 2007). Moreover, the research results from Popova (2017) indicate that probiotics generate changes in growth parameters associated with improvements in physicochemical characteristics and meat quality.

Based on this background, the objective of this study was to evaluate the effect of lamb diets with increasing levels of *Saccharomyces cerevisiae* on productive animal performance, apparent digestibility, and physicochemical characteristics of the muscle under intensive conditions.

2. Material and Methods

The experimental protocol followed the specifications of the regulation for the use and care of animals destined for research (CP 02.11.16).

2.1. Location

The study was conducted in Texcoco, State of Mexico, Mexico (19°27'49.59" N, 98°54'19.92" W, 2250 m altitude). The experiment lasted 73 days, of which 15 days were an adaptation period.

2.2. Animals and treatment

We used 32 male Hampshire lambs, with an initial live weight of 25.82±1.95 kg. The lambs were de-wormed (Closantil® 5%, 1 mL 5 kg^{-1} live weight: oral, and Ivomec® 1%, 1 mL 50 kg^{-1} live weight: subcutaneously), administered vitamins (Vigantol® ADE, 2 mL $lamb^{-1}$: intramuscularly), vaccinated (Bobact® 8, 2.5 mL $animal^{-1}$: intramuscularly), and distributed in one of four treatments in a completely randomized design. Four treatments were evaluated: basal diet (80% balanced feed Engorda Cordero Plus® and 20% alfalfa hay) with the inclusion of 0, 3, 5, and 10 g $lamb^{-1}$ d^{-1} of *Saccharomyces cerevisiae* (Yea-Sacc^{1026}, Alltech de México), with eight lambs per treatment. Lambs were housed in individual metabolic cages equipped with feeders and water troughs. Feed was offered twice (09:00 and 18:00 h), and adjusted according to body weight (3%). Water was offered *ad libitum*.

2.3. Lamb performance, digestibility, and carcass sampling

Dry matter intake was assessed as weight of offered feed minus rejected feed. Daily weight gain was obtained by weighing the lambs on three consecutive days, at the beginning of the experiment and then every 15 days, in the morning before feeding. Feed conversion was calculated as the ratio between the amount of feed consumed and live weight gain.

Apparent digestibility of dry matter (DM), organic matter, neutral detergent fiber (NDF), acid detergent fiber (ADF), and total nitrogen were determined by the method of acid insoluble ash (Geerken et al., 1987), through the collection of feces, during the three days before the end of the experiment.

Samples of ruminal fluid were collected from five lambs per treatment with an esophageal tube, one day before concluding the experiment (day 57), 4 h after the morning feeding. Sample pH was measured immediately with a portable potentiometer (Orion, USA).

At the beginning and on day 55 of the experiment, dorsal fat thickness and the *longissimus dorsi* muscle area (between the 12th and 13th ribs) were measured using ultrasound Sonovet 600 equipment (Universal Medical System, USA) with a 7.5 Mhz transducer.

At the end of the experiment (day 58), five lambs per treatment were sacrificed. The hot carcass was weighed to calculate carcass yield as described by Gómez-Gurrola et al. (2017). The *longissimus dorsi* muscle was then extracted, and within the first 30 min *post-mortem*, pH was measured with a portable potentiometer for meat (Hanna, USA) and color parameters (*L**, *a**, and *b**) were determined using a colorimeter (Minolta CR-400, USA) with a chromatic system. The samples were transported on ice (4 °C) to the laboratory, and 2 h after the sacrifice of the lamb, muscle water holding capacity was measured (Guerrero Legarreta et al., 2002). The rest of the muscle samples were preserved at −20 °C until their chemical analysis.

2.4. Chemical analysis

Representative samples of feed, refusal, and feces were dehydrated in a forced-air oven (Riossa, USA) at 50 °C during 24 and 36 h, respectively, and processed in a Wiley mill (USA) with a 1-mm screen. For the chemical analysis of muscle, 50 g of tissue was dehydrated in a lyophilizer (Labconco, USA) by placing the samples in 120-mL vials previously weighed. In the feed samples, *Saccharomyces cerevisiae*, feces, and muscle tissue, we determined (AOAC, 2005) DM (method 930.15), total protein (method 984.13), and ash (method 942.05) contents. Neutral detergent fiber and ADF were determined in feed and feces (Van Soest et al., 1991). Alpha amylase and sodium sulfite were used for NDF analysis in feed and feces samples, respectively, according to the method recommendations. Ether extract samples were determined in feed, *Saccharomyces cerevisiae*, and muscle tissue according to the methodology described by Nielsen (2010). The chemical profile obtained with the different samples is presented in Table 1.

Table 1 - Chemical characterization of the diet and *Saccharomyces cerevisiae*

Component (%)	Basal diet[1]	YS1026[2]
Dry matter	97.57	95.64
Crude protein	15.93	28.4
Neutral detergent fiber	29.05	30.18
Acid detergent fiber	14.90	17.15
Ether extract	1.77	5.18
Ash	9.55	6.66
Acid insoluble ash	0.03	0.20
Metabolizable energy (Mcal kg^{-1})	2.8	-

[1] Lamb feed (Cordero Plus®, Grupo Unión, Tepexpan, 80%) + 20% chopped alfalfa.
[2] Yeast *Saccharomyces cerevisiae*.

2.5. Statistical analysis

Data were analyzed with PROC GLM of SAS (Statistical Analysis System, version 9.4). Normality of the variables was tested with the Shapiro-Wilk test, and orthogonal polynomials contrasts were run to test

the linear and quadratic effect of the levels of *Saccharomyces cerevisiae* inclusion (Steel et al., 1997). A P≤0.05 was considered statistically significant. The model used was

$$Y_{ij} = \mu + \tau_i + e_{ij},$$

in which Y_{ij} is observation j in treatment i, μ is the mean value, τ_i is the fixed effect of treatment, and e_{ij} is the error.

3. Results

Inclusion of *Saccharomyces cerevisiae* in the diet modified (P<0.05) productive performance of lambs (Table 2). The addition of 3 and 5 g d^{-1} *Saccharomyces cerevisiae* in the diet improved (P = 0.005; linear effect) feed conversion by 25.2 and 26.9%, respectively, compared with the control group. The above levels (3 and 5 g d^{-1}) of *Saccharomyces cerevisiae* led to increments in daily weight gain and final weight of 3.3 and 4.5% (P = 0.008) and 2.3 and 2.1% (P = 0.045) when *Saccharomyces cerevisiae* inclusion levels increased from 0 to 3 and 5 g d^{-1}.

In contrast, the highest *Saccharomyces cerevisiae* (10 g d^{-1}) dose reduced dry matter intake by 16% (P = 0.0008) when compared with the control treatment, and even when feed conversion was the best among the treatments, daily weight gain decreased 10.3% (P = 0.008) relative to the control group. No changes in the digestibility coefficients of the diet or in ruminal pH were observed with the addition of *Saccharomyces cerevisiae* (P>0.05, Table 3).

The addition of *Saccharomyces cerevisiae* to the diet did not affect (P>0.05; Table 4) the *longissimus dorsi* muscle area, dorsal fat thickness, hot carcass weight and yield, pH, or the chemical profile and physical-chemical characteristics of the muscle.

Table 2 - Productive performance of lambs (n = 8) fed diets supplemented with different levels of *Saccharomyces cerevisiae*[1]

Variable	*Saccharomyces cerevisiae* (g d^{-1})				SEM	P-value	
	0	3	5	10		L	Q
Initial weight (kg)	25.8	26.1	25.8	25.5	0.344	0.756	0.618
Final weight (kg)	44.6	45.6	45.6	42.5	0.522	0.145	0.045
Dry matter intake (kg d^{-1})	1.7	1.7	1.7	1.5	0.030	0.0008	0.041
Daily weight gain (g d^{-1})	326	336	340	292	5.962	0.046	0.008
Feed conversion ratio	7.7	5.7	5.6	5.5	0.278	0.005	0.062

[1] Yea Sacc[1026], Alltech.
SEM - standard error of the mean; L - linear effect; Q - quadratic effect; n - number of animals evaluated.

Table 3 - Apparent digestibility (n = 7) and ruminal pH (n = 5) in lambs fed diets supplemented with different levels of *Saccharomyces cerevisiae*[1]

Variable	*Saccharomyces cerevisiae* (g d^{-1})				SEM	P-value	
	0	3	5	10		L	Q
Dry matter (%)	81.4	81.5	81.2	79.2	0.845	0.396	0.566
Organic matter (%)	78.9	79.0	78.7	76.5	0.958	0.396	0.566
Neutral detergent fiber (%)	68.6	66.5	67.0	62.9	1.414	0.208	0.717
Acid detergent fiber (%)	67.2	63.8	64.1	60.1	1.642	0.167	0.924
Total nitrogen (%)	78.9	80.2	80.0	77.8	0.965	0.696	0.383
Ruminal pH	6.7	6.5	6.6	6.7	0.055	0.860	0.384

[1] Yea Sacc[1026], Alltech.
SEM - standard error of the mean; L - linear effect; Q - quadratic effect; n - number of animals evaluated.

Table 4 - Physicochemical characteristics of the carcass and muscle of lambs (n = 5) fed diets supplemented with different levels of *Saccharomyces cerevisiae*[1]

Variable	*Saccharomyces cerevisiae* (g d^{-1})				SEM	P-value	
	0	3	5	10		L	Q
HCW (kg)	23.2	23.2	22.4	23.3	0.295	0.810	0.478
HCY (%)	53.9	52.5	51.4	53.6	0.649	0.736	0.215
LMA (cm^2)	12.0	12.1	12.0	11.2	0.179	0.100	0.245
FT (mm)	3.9	4.1	4.0	3.9	0.071	0.847	0.203
Moisture (%)	73.4	73.5	73.2	73.6	0.344	0.956	0.821
Protein (%)	19.8	20.2	20.3	20.0	0.248	0.878	0.489
EE (%)	4.3	3.7	3.6	4.0	0.199	0.557	0.274
Ash (%)	1.2	1.3	1.3	1.2	0.035	0.755	0.281
pH	6.3	6.2	6.1	6.3	0.098	0.968	0.382
WHC	0.3	0.3	0.3	0.3	0.011	0.320	0.624
*L**	32.8	31.8	32.0	33.0	0.557	0.887	0.403
*a**	17.0	16.7	16.3	17.1	0.299	0.960	0.434
*b**	3.0	3.9	2.8	2.9	0.207	0.539	0.386

[1] Yea Sacc[1026], Alltech.
HCW - hot carcass weight; HCY - hot carcass yield; LMA - *longissimus dorsi* muscle area; FT - fat thickness; EE - ether extract; WHC - water holding capacity; *L** - brightness; *a** - red coordinate; *b** - yellow coordinate; SEM - standard error of the mean; L - linear effect; Q - quadratic effect; n - number of animals evaluated.

4. Discussion

Growth response of livestock species depends mainly on feed intake and nutrition strategy. Nutrition strategy considers the nutritive value of the feed, forage:concentrate ratio, diet protein content, and use of feed supplements such as *Saccharomyces cerevisiae*. The kind and quantity of the yeast used are decisive (Domínguez-Vara et al., 2009; Mousa et al., 2012).

The productive response of lambs to *Saccharomyces cerevisiae* supplementation varies depending on the level of yeast inclusion. Obeidat (2017) reported that 0.5 g *Saccharomyces cerevisiae* did not affect daily weight gain, while Cömert et al. (2015) observed increases in daily weight gain with 4 g of yeast, as shown in our study.

Improvements in daily weight gain associated with *Saccharomyces cerevisiae* in lambs fed a concentrated diet would be related to an increase in the flow of bacterial protein available to the small intestine (Fereli et al., 2010; Khan et al., 2016; Zicarelli et al., 2016).

Haddad and Goussous (2005) reported a higher daily weight gain in lambs that received 3 g d^{-1} *Saccharomyces cerevisiae*, and this variable was related with increases in the digestibility coefficients of the organic matter, nitrogen, and neutral detergent fiber. The better feed conversion in our study related to *Saccharomyces cerevisiae* supplementation could indicate a higher feed digestibility and greater efficiency of nutrient utilization (Rodrigues et al., 2013; Elghandour et al., 2014; Arowolo and He, 2018). However, our results did not show differences in digestibility coefficients of the diet. Two factors could explain this: the quality and nature of the forage and forage inclusion level in the diet. Regarding the first factor, the forage used in our study (alfalfa hay), a legume, contains NDF that is more digestible than the NDF of Gramineae (Oba and Allen, 1999). Chaucheyras-Durand et al. (2012) pointed out that forages with higher levels of lignin and lower levels of easily digestible carbohydrates degrade better in the presence of *Saccharomyces cerevisiae*. Moreover, the NDF of feed concentrates is generally more digestible than the NDF of forages (Cruz and Sánchez, 2000). The second factor refers to the percentage of forage (20%) in the diet of our study. Sartori et al. (2017) stated that inclusion of 30 to 50% forage favors *Saccharomyces cerevisiae* activity in the ruminal environment, coinciding with Mousa et al. (2012), who observed higher digestibility of DM, protein, and fiber when forage was 40% of lamb diet and 5 and 7.5 g *Saccharomyces cerevisiae* was added.

Regarding ruminal pH, the similarity among treatments did not suggest an effect of *Saccharomyces cerevisiae* on ruminal acidosis, a syndrome caused by ingestion of high quantities of rapidly fermentable carbohydrates (Calsamiglia et al., 2012). *Saccharomyces cerevisiae* prevents this syndrome by competing with lactic acid-producing bacteria for fermentable carbohydrates (Chaucheyras-Durand et al., 2008).

Although the inclusion of 3 and 5 g *Saccharomyces cerevisiae* improved productive performance, the response observed with higher doses (10 g d^{-1}) was not clear. Yeast cultures are fermented products that contain live yeast, the culture medium for growth, and secondary metabolites produced during fermentation (Linn and Raeth-Knight, 2006), such as phenolics, isoprenoids, alkaloids, and polyketides (Siddiqui et al., 2012). Therefore, by increasing the inclusion level of *Saccharomyces cerevisiae*, the amounts of secondary metabolites supplied increases. These secondary metabolites are regulating molecules with nutraceutical potential (Das and Das, 2015) that coordinate the cell activity of a population of one-celled microorganisms (Davies, 2010). Nevertheless, the type of secondary metabolites and high inclusion doses could have an adverse effect on animal performance (Forbey et al., 2009). Tannins are phenolic compounds that form complexes with digestive enzymes, affect rumen fermentation, and depress feed intake; alkaloids cause ataxia and diarrhea that decrease animal performance (Attia-Ismail, 2015); and isoprenoids (also known as terpenoids) are responsible of hemolysis and neurological problems (Torres-Acosta et al., 2008). The above cause weight loss, organ failure, altered metabolic rates, reduced nutrient digestibility, or changes in energy expenditure (Forbey et al., 2009).

Final lamb weight largely explains the changes in dorsal fat thickness, *longissimus dorsi* muscle area, and hot carcass yield (Hernández-García et al., 2015). However, the variations in final lamb weight by the effect of *Saccharomyces cerevisiae* supplementation (Table 2) did not modify the mentioned variables.

Lamb age is one factor that affects the linear and positive deposition of fat on the carcass (Bueno et al., 2000). In relation to the results observed in our study, the lambs were sacrificed at 130 days old, sufficient to achieve a homogeneous distribution of fat on the carcass allowing the desirable cover and quality (Issakowicz et al., 2013).

There is an inverse relationship between fat cover and meat protein content (Rufino et al., 2013). The evaluated yeast treatments, however, did not change protein neither fat content (Table 4), which in our study were within the average values reported for lamb meat (75% moisture, 9% total protein, 4% fat, and 1% mineral matter; Prata, 1999). Inclusion of *Saccharomyces cerevisiae* in concentrated diets is related to increases in intramuscular fat deposition; it favors marbling and carcass quality and improves sensorial properties of the meat (Campo et al., 2003; Hascik et al., 2009). In our study, however, the inclusion of *Saccharomyces cerevisiae* did not affect the percentage of ether extract (intramuscular fat), which is affected by factors such as breed, sex, age, and diet (Bueno et al., 2000; Partida de la Peña et al., 2013).

The lack of significant effects of *Saccharomyces cerevisiae* on color components (*L**, *a**, and *b**) and water holding capacity of lamb muscle contrasts with other studies. *Saccharomyces cerevisiae* increases water holding capacity, explained by increased water molecules linked to muscular proteins in response to a higher total protein percent in the meat (Colmenarez et al., 2014). Water holding capacity increases the diameter of muscle fibers, which causes a more open myofibrillar structure with a lower light-reflective capacity of the meat surface, generating darker colors related to increases in the value of the *a** component (red index) (Mileswki and Zaleska, 2011).

5. Conclusions

Inclusion of 3 and 5 g $lamb^{-1}$ d^{-1} *Saccharomyces cerevisiae* in concentrated diet for lambs improves productive efficiency to reach slaughter weight in less time due to greater weight gain and better feed conversion. Nevertheless, none of the *Saccharomyces cerevisiae* levels evaluated affects the physicochemical characteristics of the carcass or muscle.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: A. Gloria-Trujillo, D. Hernández-Sánchez and M.A. Mata-Espinosa. Data curation: O. Hernández-Mendo. Formal analysis: D. Hernández-Sánchez and O. Hernández-Mendo. Funding acquisition: R. Pinto-Ruiz and A.I. Osorio-Teran. Investigation: A. Gloria-Trujillo and M.A. Mata-Espinosa. Methodology: D. Hernández-Sánchez and M.M. Crosby-Galván. Project administration: A. Gloria-Trujillo and D. Hernández-Sánchez. Resources: D. Hernández-Sánchez, M.A. Mata-Espinosa and A.I. Osorio-Teran. Software: M.A. Mata-Espinosa and R. Pinto-Ruiz. Supervision: M.M. Crosby-Galván, R. Pinto-Ruiz and A.I. Osorio-Teran. Validation: M.A. Ayala-Monter and A.I. Osorio-Teran. Visualization: M.M. Crosby-Galván and M.A. Mata-Espinosa. Writing-original draft: A. Gloria-Trujillo, O. Hernández-Mendo, R. Pinto-Ruiz and M.A. Ayala-Monter. Writing-review & editing: D. Hernández-Sánchez and M.A. Ayala-Monter.

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