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RESEARCH ARTICLE

Effect of orange pulp fermented in solid substrate with *Saccharomyces cerevisiae* in diets, on productive behavior of confined lambs

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ABSTRACT

The effect on growth performance of diets containing orange pulp fermented in solid substrate with the yeast *Saccharomyces cerevisiae (S. cerevisiae)* was evaluated in feedlot lambs. Fifteen non-castrated male lambs (Pelibuey x Dorper) with an initial weight of 22 ± 4 kg were distributed in individual pens in a completely randomized design. The feeding trial lasted 60 days. Treatments were: treatment 1 (T1), 50% commercial feed plus 50% wet basis (WB) of orange pulp fermented with yeast; treatment 2 (T2), 50% commercial feed plus 50% (WB) fresh orange pulp without yeast; and treatment 3 (T3) 100% commercial feed as a total mixed ration. The variables of the study were: average daily weight gain, feed consumption, feed conversion ratio, area of *Longissimus dorsi* and back fat thickness, the latter two measured at the end of the test using ultrasound. Daily weight gain was significantly lower ($P<0.05$) in lambs of T2 compared with those of T1 and T3 which had similar ($P>0.05$) weight gain; average daily gain (ADG) for T1, T2 and T3 was 0.270±0.027, 0.200±0.035 and 0.273±0.038 kg/d, respectively. Feed conversion ratio (feed consumed/weight gain) of lambs in T2 presented worth value ($P<0.05$) than those of T1 and T3. There was no difference in *Longissimus dorsi* area or back-fat thickness ($P>0.05$). In conclusion, substituting orange pulp fermented in solid state with *S. cerevisiae* for 50% of a commercial diet resulted in similar productive behavior of confined sheep, whereas substitution with non-fermented orange pulp may reduce growth performance of lambs.

Keywords: Citrus-pulp; Fermentation; Sheep; Waste-byproducts; Yeast.

INTRODUCTION

To solve the protein-rich feeds problem for animal feeding, it has been necessary to find new likely sources of protein-rich feeds to totally or partially replace the common protein sources in diets for animals (Chacón, 2004; Ritala et al., 2017, de Evan et al., 2020). The protein obtained by a fermentation process is called bio-protein or single cell protein (SCP), where substrates are inoculated with bacteria, fungi or yeasts (Giraldo and López, 2008; Fátima-Araújo et al., 2009), and subsequent to its fermentation will contain higher protein percentage (Giraldo and López, 2008). Yeasts are microorganisms widely used in the industry. *Saccharomyces cerevisiae* is the most scientifically studied; it is attractive for commercial production as the protein is easy to replicate and has no relationship with pathogens of risk for humans (Fátima-Araújo et al., 2009; Gloria et al., 2014; Shurson, 2018).

The citrus by-products are added to animal feed as an ingredient in fresh or dried form (de Evan et al., 2020). It's still the simplest and cheapest way to dispose of large amounts of waste produced from citrus processing facilities every day (Chavan et al., 2018). Citrus by products contain the peel, pulp, rag, and seeds and are isolated by solid liquid separation during the juice production process. Although citrus by-products are sometimes called citrus waste, they still contain large amounts of valuable compounds, such as fiber, protein, pectin, polyphenols and essentials oils (Chen et al., 2019). Agro-industrial waste byproducts can be fermented for bio-protein production (Dúran, 1989) and for the development of new compounds with commercial

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value (Butolo, 1996; Cypriano et al. 2018). The waste byproducts must contain high percentage of carbohydrates to be broken down by microorganisms (Díaz et al., 2010). Agro-industrial waste by-products include fruits and vegetables such as banana peel, cassava, corn, sugar cane, apple, molasses, stillage, wheat, chickpeas, coffee pulp, boiled rice, lemon peel, passion fruit, pineapple, guava, strawberry, orange, among others (Correia et al., 2007; Chen et al., 2019); these substrates should contain approximately 70% moisture (Yang, 1988) and may require processing previous to inoculation for greater availability of nutrients needed during the fermentation process (Raimbault, 1998; Giraldo and López, 2008).

In solid-state fermentation, *Saccharomyces cerevisiae* has increased the nutritive value of cactus pear (Araújo et al., 2008) potato peels (Maxwell et al., 2019) apple pomace (Joshi and Sandhu, 1996) pineapple (Correia et al., 2007) lemon pulp (Morovat et al., 2014; Dadvar et al. 2015) and orange pulp (Sayed, 2018). According with Sharma et al. (2017) citrus crops are copious in tropical and subtropical regions; high productions are obtained in Brazil, United States, China, Mexico and the European Union Countries. The enormous amounts of wastes generated by the industrial citrus-processing (without proper processing) produce environment problems. Orange pulp is an agro-industrial by-product of relatively low cost and its chemical composition favors its use when treated with microorganisms (Chen et al., 2019).

Interesting, there are limited publications on the use of orange pulp treated with *Saccharomyces cerevisiae* in solid state fermentation and considering evaluations on the growth performance of small ruminats. Sayed (2018) observed improved ADG in lambs at replacing 40% of treated orange pulp with *Saccharomyces cerevisiae* by the concentrate in the diet; in this study was not considered a treatment with fresh orange pulp. Our hypothesis was that productive performance of finishing lambs is improved when fed orange pulp fermented in solid state with *Saccharomyces cerevisiae* compared untreated orange pulp. The objective of this this study was to assess the productive performance of confined lambs (*Ovis aries*) in the growing-finishing stage when fed diets with orange pulp fermented in solid state with *Saccharomyces cerevisiae*, considering 50% total mixed ration replaced by treated or untreated orange pulp.

MATERIALS AND METHODS

Research site

This research was carried out at the College of Veterinary Medicine and Animal Science "Dr. Norberto Treviño Zapata", Autonomous University of Tamaulipas, in Ciudad Victoria, Tamaulipas, Mexico. Which is geographically located at 23° 44' 06" North and 97° 09' 50" West, at an altitude of 340 meters above sea level, with an average temperature of 25°C and 900 mm of average yearly precipitation; these conditions are typical for dry tropics (INEGI, 2006).

Orange pulp, collection and inoculation

The orange pulp was the residue obtained after the juice extraction, and it was acquired from two shops dedicated to the sale of orange juice in Cd. Victoria, Tamaulipas, México. The oranges were of Valencia variety used for juice, and are commonly produced in the central region of Tamaulipas. In the process, the raw material (orange pulp) was heated with steam for 20 minutes to soften the plant tissue, and then was ground using an industrial blender to obtain small and homogeneous particles.

Once crushed, the orange pulp was cooked for 30 min at 90°C, and was left to drain and cooling in a mesh for one hour; after, 55 g of yeast were directly added per 2.27 kg of orange pulp. The inoculum used was the yeast *Saccharomyces cerevisiae* (Tradipan®) acquired in 11-g envelopes (6 x 10^8 CFU/g). The solid substrate fermentation was performed in 20-liter plastic buckets, stirring the yeast with the substrate, which had at least 80% humidity. Afterwards, the buckets were left for 48 hours at room temperature. The product obtained after fermentation was used for supplementation of lambs.

Chemical analysis

Orange samples were dried at 60°Cfor 48 h and then with a stove at 105°C for 12 h. Samples were analyzed for total ashes, ether extract (EE) and crude protein (CP) according to the procedures of the Association of Official Analytical Chemist (AOAC, 1996).

Animals, treatments and feeding management

The study was carried out in accordance with the Committee of Bioethics and Animal Care of the College of Veterinary Medicine and Animal Science, Autonomous University of Tamaulipas. Fifteen non-castrated crossbred (Pelibuey x Dorper) male lambs were used for this study. They were treated against parasites (Closantil® 5%; Closantel 50 mg/ml [CHINOIN®]), vaccinated with triple action bacterin (Bacterina Triple C.E.S. ® [INTERVET®] and injected with fat-soluble vitamins A, D and E (Vigantol ADE® ; A 500.000 U.I., D3 75.000 U.I., E 50 mg. in 1 ml. [Bayer®]). The lambs were given 15 days of adaptation, and were housed indoors in individual pens with concrete floor and wood shavings as litter. At the start of the test, the average weight was 22 ± 4 kg and lambs had 135 ± 10 d of age. The lambs were in the growing-finishing stage, and were confined into individual pens of 120c m long x 70 cm wide x 100 cm high, with individual drinkers and 30-cm feeders. Water was given *ad libitum*. Lambs were separated into 3 groups of 5 animals each; the experimental unit was the animal. Group 1 (treatment 1) was fed with 50% total mixed ration (TMR) and 50% of DM of the orange pulp treated with yeast; treatment 2 was fed with 50% of TMR and 50% of yeast-free fresh orange pulp, and treatment 3 received 100% TMR. The total mixed ration is commercial feed used in sheep fattening. The TMR is formed by cereal grains, oilseed meals, sugarcane molasses, forage and minerals. The TMR is formulated at minimum of 13% of crude protein. Feed was offered twice a day at 9:00 and 17:00 hours in equal amounts. The offered feed was adjusted daily according to consumption, and was 10% more than that consumed, which was calculated based on feed offered minus refused. The weighing of animals was carried out between 10:00 and 11:00 hours at 15-day intervals for a total period of 60 days. The animals were fasted of 12 hours prior to weighing.

Fed intake and weight gain, average daily gain

Feed intake was determined using the difference between the feed offered and that rejected during 24 h. Daily weight gain was measure by weighing animals on days 0, 15, 30, 45 and 60 of the trial. Average daily gain was estimate for each 15-day period. Average daily gain (ADG) was determined by dividing the weight gain (final live weight (LW) - initial LW) by the number of days (n= 60 days). Feed conversion was calculated as the ration between DMI and ADG (g of DMI/g of LW gain).

Measurement of backfat thickness and *Longissimus dorsi* area

Carcass characteristics are evaluated in some growth performance tests, since the size of the cuts and fat content are factors that influence meat acceptance among consumers; consequently, *Longissimus dorsi* area and back fat thickness are measured with real time ultrasound in live animals (Silva et al., 2015). Measurements of the *Longissimus dorsi* area and back fat thickness were taken in the intercostal space between the 12th and 13th ribs (Silva et al., 20015), using ultrasound equipment (Aloka SSD-500) and a 7.5-MHz external use transducer at the end of the trial. An area of approximately 25 cm² was prepared by cutting the hair and shaving the skin. During the measurement the transducer was covered with a nylon bag to which vegetable oil was applied to allow ultrasound transmission. The image was frozen and with the aid of ultrasound software the area of the *Longissimus dorsi* (cm²) and the thickness of the back fat layer (mm) were determined.

Experimental designs and analysis statically

The experiment lasted 60 days, and a completely randomized design was used, with 3 treatments and 5 repetitions. The variables studied were: feed consumption (recorded daily), weight gain (recorded every 15 days), feed conversion, back fat thickness and *Longissimus dorsi* area. Data were subjected to one-way analysis of variance (ANOVA), using a significance of $P < 0.05$. For variables with statistical significance, the means were separate using the least significant difference test according with the procedures of Steel and Torrie (1988). Statistical estimations were performed using STATISTICA 6 software.

RESULTS AND DISCUSSION

Chemical composition

Chemical composition was determined for both, untreated orange pulp and pulp fermented with *S. cerevisiae* at 48 hours of incubation (Table 1). Orange pulp waste was similar to those described by Chen et al. (2020), but an increase in protein concentration was observed for the fermented orange pulp; similar results were described by Joshi and Sandhu (1996) with apple pulp used for juice production. These results are consistent with others *Saccharomyces cerevisiae* in solid state fermentation with waste byproducts of pineapple (Correia et al. 2007), lemon pulp (Morovat et al., 2014; Dadvar et al. 2015) and orange pulp (Sayed, 2018). However, the resulting values of microbial protein are not homogeneous, since there are various factors that interact to produce higher or lower increases in protein concentration; among these factors are the viability and type of strain or whether the substrate characteristics (moisture, carbohydrates) as has been pointed out in several studies (Titi et al*.,* 2008; Sales, 2011; Gloria et al*.,* 2014).

Chemical composition of diets is presented in Table 2. Orange pulp generated higher crude protein due to fermentation with *S. cerevisiae*. This results are consistent with others studies reported by Chacón, (2004); Giraldo and López, (2008) and Fátima-Araujo, (2009), who observed that the use of new biotechnologies based on the use of microorganisms such as yeasts is feasible for the increase of protein in low-protein substrates.

Table 1: Chemical composition of orange pulp with or without 48-hour fermentation (Dry Matter basis, %).

Composition	Non-fermented orange pulp	Orange pulp fermented with *Saccharomyces cerevisiae*
Dry Matter	25.63	17.47
Crude Protein	6.87	8.79
Ether Extract	1.98	1.87
Ash	5.13	5.55
Crude Fiber	13.58	14.22
Nitrogen-Free Extract	72.44	69.57

Feed intake and live weight gain

Feed intake and live weight gain data are present in Table 3. Feed intake of lambs was not affected by treatments (P > 0.05), results consistent with research conducted by Sayed et al. (2018) feeding lambs in diets with orange pulp with Saccharomyces cerevisiae.

Average daily gain (ADG) was similar (P>0.05) for T1 and T3 with 270 and 273 g/d, respectively, and different (P < 0.05) from T2 with 200 g/d. Also, Sayed (2018) observed improved ADG in lambs fed diets with orange pulp treated with *Saccharomyces cerevisiae*. The improved productive performance could be associated with increased crude protein concentration in treated orange pulp compared with untreated orange pulp, also could be increased coefficient digestibility of nutrients. Dadvar et al. (2015) reported improved percentage of crude protein and digestibility (goat study) of NDF and crude protein when lemon pulp was treated with *S. cerevisiae* compared with untreated citrus pulp.

Miranda-Yuquilema et al. (2018) used agro-industry wastes treated with bacteria and yeast to produce preparations with probiotic effect. In current research is not possible to establish the probiotic activity of yeast in the *S. cerevisiae* treatment. Further research in this aspect is warranted; however, the yeast has improved growth of lambs through the ruminal digestion increase of fiber-rich feeds (Ruiz-Barrera et al., 2020). The increase in rumen fiber degradation with yeast is consistent with others (Cömert et al. 2015; Chaucheyras-Durand et al., 2015). The productive performance improvement with yeast, also is consistent with others (Haddad and Goussous, 2005; Titi et al. 2008; Jia et al. 2018).

Table 2: Chemical composition of the treatment diets used in the trial (Dry matter basis, %).

	Treatment 1	Treatment 2	Treatment 3
Composition	orange pulp + *S. cerevisiae*	Fresh orange pulp	Total mixed ration
Dry Matter	48.5	37.34	17.47
Crude Protein	12.61	10.18	13.15
Ether Extract	1.76	2.53	2.22
Ash	6.2	5.29	5.66
Crude Fiber	17.81	10.71	14.17
Nitrogen-Free Extract	61.62	71.29	64.8

Feed conversion

The effect of the fermented orange pulp on feed conversion is show in Table 3. Feed conversion efficiency of lambs fed in treatment 2 was lower (P < 0.05) than that observed in treatments 1 and 3 which statistically similar (P > 0.05). The improved feed conversion ratio in lambs fed citrus pulp treated with *Saccharomyces cerevisiae* compared with lambs fed the diet with fresh orange pulp was evident. As previously mentioned, in this study is not possible establish if the effect was due to the direct improvement of *Saccharomyces cerevisiae* on orange pulp, or it was due to the probiotic effect of the yeast. The improvement in feed conversion ratio with yeast supplementation is consistent with others (Haddad and Goussous, 2005; Ruiz-Barrera et al. 2020). In the present study, treatment 3 was a conventional diet of commercial feed (total mixed ration) for fattening lambs and treatment 1 was a diet substituting the commercial feed by the product using by-products of orange fermented with yeasts; both treatments led to similar productive performance of lambs.

Measurement of back fat thickness and Longissimus dorsi area

Back fat thickness was not affected (P > 0.05) by treatments (Table 3); in a study (Ruiz-Pereyra, 2014) that included yeast and *Lactobacilli* in liquid for finishing lambs, differences in weight gain were observed, but there were no changes in the thickness of back fat, comparable to the results obtained in this research. Also Gloria et al. (2014) with the addition of yeast in the diet of lambs did not observe increase of dorsal fat in treated animals.

The *Longissimus dorsi* area was similar (P > 0.05) between treatments (Table 3). Consistent with the present work, in the study by Ruiz-Pereyra (2014), which included yeast and lactobacilli in diets for finishing lambs, there were no modifications in *Longissimus dorsi* area. Similarly, in another study that evaluated the growth and carcass quality of steers fed high forage diets plus concentrate, the inclusion of *S. cerevisiae* did not affect *Longissimus dorsi* area (Gloria et al., 2014).

Table 3: Effects of diet treatments on growth performance in confined sheep.

Variable	Treatment 1 orange pulp + *S. cerevisiae*	Treatment 2 Fresh orange pulp	Treatment 3 TMR	SEM
Average daily gain (kg)	0.270±0.027[a]	0.200±0.035[b]	0.273±0.038[a]	0.015
Average feed consumption (kg)	2.112±0.251	2.135±0.373	1.907±0.233	0.131
Feed conversion	7.815±0.430[b]	10.836±2.043[a]	7.023±0.779[b]	0.575
Back fat thickness (cm)	0.634±0.016	0.601±0.075	0.636±0.044	0.023
Longissimus dorsi area (cm^2)	9.539±0.717	9.449±0.929	9.914±1.068	0.410

[ab]different superscripts in a row are significantly different (P < 0.05)
TMR. Total mixed ration
SEM. Standard error of the mean

CONCLUSION

The addition of the yeast *Saccharomyces cerevisiae* to ferment orange pulp increased protein levels compared to non-fermented pulp. The substitution of 50% of a total mixed ration with orange pulp fermented with *Saccharomyces cerevisiae* resulted in similar growth performance in confined sheep; however, a similar substitution with non-fermented orange pulp leads to reduced growth performance in sheep.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authors' Contributions

Nadia Alejandra Sánchez-Guerra: Conceptualization, Investigation, Supervision, Writing - review & editing. **Miguel Ángel Domínguez-Muñoz:** Supervision, Writing - review & editing. **Miguel Ruíz- Albarrán:** Formal analysis, Writing - review & editing. **Rigoberto López-Zavala:** Conceptualization, Investigation. **Fidel Infante-Rodríguez:** Investigation, Supervision. **Luis Manuel Pérez-Quilantán:** Conceptualization, Investigation, Supervision, Funding acquisition. **Jaime Salinas-Chavira:** Formal analysis, Writing - review & editing and drafted the manuscript.

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