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Effect of Selenium-Enriched Yeast Supplementation on Microbial Spoilage and Lambs' Meat Quality during Shelf Life

Research Article

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ABSTRACT

The aim of the current research was to evaluate the effect of selenium-enriched yeast supplementation on microbial spoilage and the biochemical quality of lamb meat during shelf life. Nine Pelibuey lambs (*Ovis aries*) were randomly assigned to one of three treatments: a control without selenium supplementation (T1), and two supplemented with selenium enriched yeast (*Saccharomyces cerevisiae* Selyeast 3000^{TM} enriched yeast, LFA Lesaffre) with either 0.35 mg/kg (T2) or 0.60 mg/kg of selenium (T3), respectively, for 60 days. Microbiological spoilage and biochemical characteristics were evaluated during 0, 3, 6, and 9 days post-slaughter under refrigeration conditions (4 ˚C). Immediately after slaughter, no significant differences were found in aerobic plate count, fecal coliforms count, psychrophiles, pH, a* (Redness), and b* (Yellowness) among treatments. However, statistical differences ($P<0.05$) were observed within treatments in Psychrophilic, pH, L*(Lightness), and b* along the storage period; pH values decreased in all groups during storage, nevertheless higher doses of Se kept the highest pH values by the end of the observation period. As expected, there was a significant positive high correlation between day and b* (rxy=0.83; $P<0.001$), and a negative high correlation between pH and b* (rxy=-0.82; $P<0.001$). Selenium antioxidant and antimicrobial effect on meat did not result as expected and it's hypothesized that an extreme care of animal and meat sanitary manipulation minimized its effect.

KEY WORDS lambs, meat color, microbial growth, storage period.

INTRODUCTION

Meat is a perishable product that must be stored under chilled conditions to reduce microorganisms' growth and other deteriorating changes that can make it unacceptable for consumers. Nowadays, chemical additives are used in the meat industry to prevent food-borne pathogen growth and extend meat shelf life (Radha *et al.* 2014). Lipid oxidation and bacterial spoilage reduce meat shelf life; therefore, some natural compounds with antimicrobial and antioxidant functions, like selenium, are being added to animal basal diets to improve production, carcass composition, and fresh meat quality and extend meat shelf life (Grashorn, 2007; Yanian *et al.* 2011; Zhang *et al.* 2020; Bai *et al.* 2022). This can be given either as a salt or as an organic compound associated with Yeast. *Saccharomyces cerevisiae* Selyeast 3000 is rich in organic selenium (Se) yeast, mainly as selenomethionine, which is highly bioavailable and has been used in the beef industry, diminishing ruminal acidosis, increasing its performance, feed conversion efficiency, and meat quality. The main objective of this study was to evaluate the selenium-enriched yeast supplementation effect on

lamb meat over microbial spoilage and biochemical characteristics (pH, lipids oxidation, and color) during shelf life.

MATERIALS AND METHODS

The current work was carried out in the company "Agrovix" at Jocotitlan Municipality, State of Mexico, Mexico, and at laboratories of the Autonomous University of the State of Mexico.

All animals used in this research were treated by the Guide for the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010), NOM-033-ZOO (1995) and NOM-009-ZOO (1994) recommendations.

Animal handling and growth

Nine Pelibuey (*Ovis aries*) breed female lambs with an initial average body weight of 27.75 ± 3.37 kg were used in the study. Animals were randomly assigned to one of three treatments (n=3 for each treatment): Control group without selenium (Se) supplementation (Se0), and two treatment groups dosed, as Selyeast 3000^{TM} recommended, with 0.35 and 0.60 mg/kg (Se35 and Se60, respectively), with selenium enriched yeast (SY) *Saccharomyces cerevisiae* Selyeast 3000^{TM} enriched yeast, LFA Lesaffre, in Toluca, State of Mexico, Mexico. Lambs were fed a balanced diet with 3.1 Mcal/kg for energy and 10.16% of crude protein per kg dry matter (NRC, 2007). The main diet's ingredients (%) were: whole grain sorghum (30.5), ground corn (10.5), cracker crumbs (20.5), rolled corn (10.0), distillers dried grains (10.0), bran (10.0), molasses (8.0), vitamins and minerals (0.5). Water and feed were offered *ad libitum*, while selenium-enriched yeast was provided as an oral individual daily dose before the first meal. Each group was allocated in a single cage. Animals were slaughtered on day 60 after initial Se-yeast supplementation in a slaughterhouse following the Mexican Official Normativity (NOM-009-ZOO, 1994; NOM-033-ZOO, 1995).

Sampling

The meat was allowed to mature at room temperature for 24 hours. Rib eye (M. longissimus thoracis) pieces were cut from the 8-rib rack from each animal and individually vacuum packed and refrigerated (2-5 ˚C) until further analysis. For Meat Shelf-life analysis, one rib eyepiece from each animal was analyzed for color, pH, microbiology content, and lipid oxidation during 0, 3, 6, and 9 days of shelf life.

Color and pH

Meat color: Lightness (L*), Redness (a*), Yellowness (b*), was measured by triplicate with a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan) and pH with an electronic pH meter (HI99163 membrane pH meter, Hanna Instruments, USA) (Honikel, 1998).

Microbiological analysis

Samples were analyzed for aerobic plate count (APC), fecal coliforms count (FCC), and psychrophile (Psc). Each sample was prepared according to NOM-110-SSA1 (1994) and 1/10 serial dilutions were prepared up to 10^{-3}.

Aerobic plate count and psychrophile bacteria

APC and Psc were determined according to NOM-092-SSA1 (1994), and the CFU/plate was counted, and values transformed to $\log_{10}$ CFU/cm^2.

Fecal coliforms count

For fecal coliform counts, 1 mL samples of homogenized meat dilutions were poured on Violet Red Bile Agar (VRBAsurface andrich Co., MO, USA) and a second layer of VRBA (1 mL) was added to cover the whole plate surface, and incubated at 45 ˚C for 24 h. CFU/plate was counted and values transformed to $\log_{10}$ CFU/cm^2 (Association Françoise de Normalization NF V08-60, 1996).

Lipids oxidation assay (TBARS test)

Meat lipid oxidation was determined by Thiobarbituric Acid Reactive Substances (TBARS) test with the kit "Bio-Assay Systems, QuantiChrom™ TBARS Assay Kit (DTBA-100)" following manufacturer's instructions and Ripoll *et al.* (2011), at 0, 5 and 9 days; results were expressed as Thio-barbituric Acid Reactive Substances (TBARS) in milligrams of malondialdehyde per kg of muscle (mg MDA/kg muscle). Duplicates (100 µL) from each sample were read in a 96 wells dish in the spectrophotometer (VersaMax, Molecular Devices) at 535 nm and analyzed with softmax software (Softmax, Molecular Devices, CA, USA) and OD values were transformed to mg MDA/kg muscle. Sample readings were compared with the standard curve to determine MDA oxidation.

Statistical analysis

Data was analyzed using a completely randomized design using each animal as an experimental unit as follows:

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:
Y_{ij}: each individual observation for a given variable.
μ: overall mean.
T_i: treatment.
E_{ijk}: residual random term.

Differences among groups and days of evaluation were determined by Tukey test (95%). Pearson correlations (r_{xy}) were calculated between variables pairs (APC, FCC, Psc bacteria, lightness(L*), redness (a*), yellowness (b*), pH

and lipid oxidation values) with a 95.0% confidence level. Stat Graphics software version 5.0 plus was employed for all statistical analysis.

RESULTS AND DISCUSSION

Statistical tests found differences within groups for Psc, pH, lightness (L*), yellowness (b*) and lipid oxidation ($P<0.05$) considering the time as a variable for evaluation (Table 1). Lipid oxidation values presented some differences within treatments and among groups. MDA mg/kg muscle values ranged from 0.16 to 0.17 on day 0 and from 0.61-0.62 on 9th day.

As expected there were significant positive correlations between day and Psc ($r_{xy}=0.79$; $P<0.001$), day and L* ($r_{xy}=0.54$; $P<0.001$), day and b* ($r_{xy}=0.83$; $P<0.001$); FCC and pH ($r_{xy}=0.35$; $P<0.03$), Psc and L* ($r_{xy}=0.61$; $P<0.001$), L* and b* ($r_{xy}=0.7$; $P<0.001$). A negative correlation between day and FCCs) ($r_{xy}=-0.42$; $P<0.01$), day and pH ($r_{xy}=-0.76$; $P<0.001$), and day and a* ($r_{xy}=-0.41$; $P<0.01$); FCC and Psc ($r_{xy}=-0.43$; $P<0.01$), FCC and b* ($r_{xy}=-0.33$; $P<0.04$); Psc and pH ($r_{xy}=-0.6$; $P<0.001$); pH and L* ($r_{xy}=-0.54$; $P<0.001$) and pH and b* ($r_{xy}=-0.82$; $P<0.001$); and between redness (a*) and lipid oxidation ($r_{xy}=-0.33$; $P<0.04$) were found.

No statistical differences were observed in meat shelf life for all studied parameters among treatments. Logically meat quality changed over time and some statistical differences ($P<0.05$) were observed for the following parameters: coliform CFU's, Psc, pH, lightness, yellowness, and lipid oxidation. Unsurprisingly Psc CFU units increased with time; however, these values remained well under the maximum values as suggested by Pivinch *et al.* (1975). These bacterial values tended to be slightly higher for the Se35 treatment compared with the other treatments (Table 1). Lightness is normally associated with larger numbers of bacteria (Kalhotka *et al.* 2013); consequently, slightly increased lightness values observed in Se35 meat samples could be related to the Psc greater bacterial growth observed for these treatments compared to the other two treatments.

On the other hand, Psc growth was favored by a neutral pH (around 7.0) (Ray and Bhunia, 2008). Se35 treatment pH values were slightly higher than the other group values. This pH value could have favored Psc growth so that the higher tendency for Psc numbers was observed, which explains the pH values found. This small increment in Psc CFU suggests that the Se35 dose used could somehow favor this growth.

We found no lipid oxidation differences among groups. MDA is an assay designed to determine lipid oxidation.

The lower the MDA/kg value analyzed, the lower the lipid oxidation meat values. Vignola *et al.* (2009) found 1.36-1.69 mg of MDA/kg in meat after a day storage period, when supplying 0.3–0.45 mg of Se-yeast/kg on feed concentrate to animals for 60 days before slaughter. According to Ripoll *et al.* (2011), the MDA acceptable maximum value should be 1mg MDA/kg of sheep meat. In the present study was observed that handling protocols can prevent lipid oxidation and keep meat samples well under MDA 1 mg/kg.

pH value is a variable that influences several quality parameters such as color, shelf life, flavor, microbiological stability, performance, and texture (Lawrie, 2006). In the present study, there was no difference between initial and final pH values among treatments were found. Present pH values decreased from 7.15 at 45 min to 5.53 at 24 hours after slaughter at all treatments. These findings agree with Vignola *et al.* (2009), who did not find pH differences between treatments when providing different levels or sources of Se supplementation on lambs' meat quality. Color (lightness (L*), redness (a*), and yellowness (b*)) is a meat quality parameter that may be indicative of animals' age and genetics, animal production system, and animal care before slaughtering. Although, it should be considered that bacterial contamination can make the meat turn lighter at first and duller, due to slime formation and then discoloration (Ellis and Goodacre, 2001).

Sheep meat optimal lightness values should be between 35.8 and 45.8 L*, any values beyond this range are considered undesirable by meat consumer associations (Ponce *et al.* 2013). In this research, there were no significant differences for L* either by treatment or by shelf life. L* average value found in this study was 37.57, higher than reported by Mariezcurrena *et al.* (2013), who found a 35.95 L* value for imported New Zealand meat, but lower than reported by Vignola *et al.* (2009) in lambs (44.63). Anyway, according to Dave and Ghaly (2011), L* values reported here lay within normal, consumer-accepted values, which is an indicator factor; moreover, genetics, animal husbandry, transportation, pre-slaughter conditions, slaughtering, meat handling, and meat shelf storage were performed with high sanitary standards in this experiment.

One of the most significant qualities in purchase decisions, associated with freshness and quality is the cherry-red color (Brewer *et al.* 2002; Djenane *et al.* 2003; Mancini and Hunt, 2005; Arbiza and Tron, 2008). There were no treatment effects for a* color during shelf-life. This project values ranged from +9.8 to +15.3 with a 13.64 $\pm$ 1.58 average values; therefore, it is reasonable to think that myoglobin was in an appropriate chemical state, indicating that the product had adequate handling during shelf-life.

Table 1 Tukey test for effect of selenium supplementation and day on microbiological profile and physicochemical characteristics during meat shelf life

Item	Levels of selenium (Se, ppm)			P-value	SEM
	Se0	Se35	Se60		
APC (log_{10} CFU/cm^2)					
Day 0	0.18	0.19	0.21	0.4865	0.19
Day 3	0.19	0.19	0.22	0.7730	0.03
Day 6	0.13	0.14	0.18	0.6883	0.04
Day 9	0.22	0.26	0.16	0.1429	0.03
P	0.0907	0.2603	0.4731		
FCC (log_{10} CFU/cm^2)					
Day 0	0.03^{x}	0.04^{x}	0.03^{x}	0.9453	0.03
Day 3	0.00^{y}	0.04^{x}	0.05^{x}	0.3759	0.02
Day 6	0.00^{y}	0.00^{y}	0.00^{y}	-	-
Day 9	0.00^{y}	0.00^{y}	0.00^{y}	-	-
P	0.0061	0.00611	0.0061		
Psc (log_{10} CFU /cm^2)					
Day 0	0.00^{x}	0.06^{x}	0.00^{x}	0.4219	0.03
Day 3	0.03^{x}	0.07^{x}	0.08^{x}	0.8382	0.06
Day 6	0.21^{y}	0.28^{y}	0.27^{y}	0.4953	0.04
Day 9	0.32^{z}	0.38^{y}	0.34^{bz}	0.0340	0.01
P	0.0000	0.0087	0.0018		
pH					
Day 0	6.15^{x}	6.30^{x}	5.96^{x}	0.0062	0.04
Day 3	5.72^{x}	5.66^{y}	5.74^{y}	0.4634	0.04
Day 6	5.34^{y}	5.12^{z}	5.46^{z}	0.1422	0.11
Day 9	5.09^{z}	5.62^{y}	5.56^{z}	0.0603	0.10
P	0.0001	0.0002	0.0001		
Lightness (L*)					
Day 0	35.88^{x}	38.93^{x}	37.91^{x}	0.1853	1.03
Day 3	37.92^{xy}	40.03^{y}	38.88^{y}	0.4939	1.18
Day 6	41.24^{y}	42.69^{y}	41.61^{y}	0.6603	1.13
Day 9	41.30^{y}	43.67^{y}	38.57^{y}	0.1750	1.66
P	0.0432	0.0449	0.04831		
Redness (a*)					
Day 0	15.30	12.40	13.27	0.0921	0.78
Day 3	12.05	12.25	14.81	0.5585	1.92
Day 6	12.03	12.28	13.55	0.8752	2.21
Day 9	9.81	10.25	10.71	0.9073	1.44
P	0.1952	0.4616	0.6202		
Yellowness (b*)					
Day 0	6.48^{x}	5.03^{x}	5.53^{x}	0.0990	0.39
Day 3	10.54^{y}	10.88^{y}	11.07^{y}	0.8865	0.77
Day 6	14.54^{z}	12.98^{yz}	11.95^{y}	0.0524	0.55
Day 9	14.19^{z}	14.17^{z}	12.27^{y}	0.5974	1.47
P	0.0019	0.0001	0.0002		
LO (TBARS) (mg MDA/ kg)					
Day 0	0.16	0.17	0.17	0.9639	0.02
Day 5	0.35	0.29	0.23	0.3687	0.05
Day 9	0.62	0.70	0.61	0.2523	0.58
P	0.3412	0.0718	0.4936		

APC: aerobic plate count; FCC: fecal coliforms count; Psc: pscychrophiles; LO: lipid oxidation and TBARS: thiobarbituric acid reactive substance.
[a, b, c] Means with different lower case letters in the same row are significantly different (P<0.05).
[x, y, z] Means with different lower case letter in the same column are significantly different (P<0.05).
SEM: standard error of the means.

Present results indicate that b* values from all the samples ranged from 5.03 to 14.54 and were maintained within acceptable lipid oxidation values; furthermore, it means that meat was stored within acceptable standards. There were no differences ($P<0.05$) found due to treatment. There was a time effect over the shelf life for the three treatments within groups, a tendency to a slightly lower lipid oxidation was observed in Se65 treatment. A lower b* value change was found (from 5.53 to 12.27 a*) in that group, as compared to the lipid oxidation accumulated by Se35 and the control group, with values starting at 5.03 and 6.48 and ending in 14.17 and 14.19 a*, respectively. It is noteworthy to point out that yellowness values are considered the best results when they are closer to zero and that average values found in this study were closer to zero similar to some previous studies (Vignola *et al.* 2009; Mariezcurrena *et al.* 2013).

According to Meadus and MacInnis (2000), glycolytic potential is correlated to L* and b* which suggests that increasing glycolytic potential would promote acidity, paleness, and yellowness. Current results are in agreement with these authors; since it has been observed that L* and b* values increased along shelf life up to 9 days. In this report, it had been found values ranging between 5 (24 h after slaughter) and 14 (9 days of shelf life). Positive values are associated with lipid oxidation (thio-barbituric acid reactive substances) during shelf life. It is considered normal to find some oxidation during meat shelf life and the values found in the present study can be considered within values found in good-quality meat. A high glycolytic potential was not recorded in meat samples studied throughout this experiment; however, considering the pH, L* and b* values obtained on day nine of shelf life, a high glycolytic potential could be inferred in all meat samples studied, which is considered normal in this storage period (Meadus and MacInnis, 2000; Mancini and Hunt, 2005). All values are considered normal for sheep meat maturation.

These correlations seem to fall within normal values since Psc can be found in food predominantly during long refrigeration storage periods. A positive correlation between day and L* was also found by Vignola *et al.* (2009) who reported that lightness (L*) increased during shelf life. It was expected; a significant positive correlation between Psychrophiles and day of shelf storage was found, *i.e.,* the bacterial load increased over time. According to Vasut (2009), Selenium (Se) has a reductive effect on Psc growth in meat samples at refrigeration temperatures (3-5 ˚C). In the current research a negative correlation between day and a* was detected. This is in agreement with observations by Vignola *et al.* (2009), who reported a reduction of redness (a*) over time, probably due to water loss and oxymioglobin to metmyoglobin oxidation. Furthermore, meat oxidation has also been associated with myofibrils water reserve loss, which increases meat juice loss and has an intense redness effect (Ripoll *et al.* 2011). Antioxidant and antimicrobial organic selenium effect on meat did not result as expected. Considering these results, it has been hypothesized that extreme animal and meat care done during the process (from animal transportation to the slaughterhouse), animal care before slaughter, and meat handling after slaughtering had a positive impact on meat quality and meat preservation over shelf life. Therefore, the putative effects of Se in sheep's diet over meat quality were masked.

CONCLUSION

It has been concluded that there was no selenium-enriched yeast supplementation positive effect on either lamb meat over microbial spoilage or biochemical quality (pH, lipids oxidation and color). Even though other studies have demonstrated Se positive effects on meat quality, further experiments on lamb meat including higher Se doses were suggested, especially for shelf-life evaluations (normal farming and slaughtering conditions).

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