

Effect of organic selenium supplementation in the diets of finishing sheep on meat color and pH during shelf life

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ABSTRACT

Supplementation of Selenium (Se) can improve the oxidative stability of meat products and retard metmyoglobin formation; so prolonging color. Effect of organic Se addition in the diets of sheep on meat stability; color and pH are limited and need to be studied. A study was conducted with eighteen Pelibuey female sheep at the finishing stage, and supplemented with organic Se-enriched *Saccharomyces cerevisiae* for sixty days to evaluate its effect on color and pH of *Longissimus dorsi* muscle. The research was conducted in a block randomized design considering three treatments; control (Se0) without the addition of yeast, or with 0.35 ppm of yeast (Se34) or with 0.60 ppm (Se59). Sheep were slaughtered at an average weight of 39.5 ± 4.41 kg. Meat color and pH were recorder in the cold carcass, 24 h after slaughtering, and during shelf life at 0, 4, 6 and 8 days after slaughtering under refrigeration at 4 °C. No significant differences were observed ($P > 0.05$) for meat color and pH characteristics due to treatment. Decreased redness (a^*) and Chroma (C^*) values due to storage time were observed; however, the yellowness (b^*) and angle Hue were increased. It could be concluded that supplementation of Se-enriched yeast in finishing sheep with 0.35 ppm and 0.60 ppm has no effect on meat color and pH characteristics.

Key words: Antioxidants, Meat quality, Meat shelf life, Se-enriched yeast, Sheep.

INTRODUCTION

The color of fresh red meat is the most important meat sensory characteristic that influences the acceptability of food and hence the likelihood of purchase. Meat color is the first quality attribute seen by the consumers who use it as indicator of freshness and wholesomeness. The oxidation of meat components during shelf life has negative effects because it affects the color and pH and therefore their appearance. It has a negative effect on purchase decisions of consumers, which result in substantial economic losses (Troy and Kerry, 2010).

Consumers prefer red meat; the red color is due to the content and form of the protein myoglobin; however, it is unstable mainly during cold storage (Jacob and Thomson, 2012). In addition, color stability is related to the amount of glycogen formed ante mortem determining the pH of meat, the trend is changing in color from red to brown after cutting and during shelf life. The brown color is mainly due to the

presence of metmyoglobin. The brown color indicates lack of freshness and quality. It depends on various factors including oxygen partial pressure and pH. The brown color is formed because myoglobin meat bright red is transformed into deoxymyoglobin purple after cutting. Moreover, when myoglobin form oxymyoglobin bright cherry red, and it is transformed into metmyoglobin when there is a low oxygen partial pressure. The color of the meat is determined by the distribution and proportion of those pigments (Mancini and Hunt, 2005; Faustman and Cassens, 1990) and can be affected by temperature, light, pH after slaughter (Abril *et al.*, 2001), lipid and protein oxidation (Li and Liu, 2012) and microbial growth (Agunbiade *et al.*, 2010).

Selenium (Se) is an essential trace element for living organisms, and its importance is mainly associated with the enzyme glutathione peroxidase, which acts as a defense against oxidative stress, protecting cell damage, avoiding lipids and proteins oxidation (Laguerre *et al.*, 2007).

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Glutathione peroxidase enzyme is found in animal's muscle when diets contain certain amounts of Se with continuous antioxidant activity in meat (Daun and Åkesson, 2004). To prevent oxidation, color stability is achieved during the shelf life, to retard metmyoglobin formation and prolong the bright red color, getting a more attractive product for consumption. There are two sources of Se for animal intake; inorganic (selenite or selenate) and organic (selenomethionine and selenocysteine). Se-enriched *Saccharomyces cerevisiae* may be considered as an organic source of Se. The organic Se-enriched yeast is assimilated and deposits better than inorganic sources (Juniper *et al.*, 2009). There are two sources of Se for animal feeding, inorganic Se in the form of sodium selenite or selenite, and organic Se as Se-enriched *Saccharomyces cerevisiae*, which transforms inorganic Se in organic natural as selenomethionine and selenocysteine, which is assimilated and deposits better than inorganic source (Juniper *et al.*, 2009). The aim of this study was to evaluate the effect of addition of Se-enriched yeast to the diet of finishing sheep on pH and color characteristics of *Longissimus dorsi* muscle.

MATERIALS AND METHODS

A total of 18 Pelibuey female sheep, with an average initial weight of 27.7 ± 3.3 kg were enrolled onto the study. Sheep were randomly allocated to one of three experimental treatments, with 6 sheep each. Sheep were fed twice daily in the morning and evening. Se-enriched yeast was individually provided to each sheep, using 20 g of whole sorghum from its diet as vehicle. The first group considered as control (Se0) only was given diet feed (0.01 ppm Se), while the second group (Se34) was given 0.35 ppm of Se-enriched yeast, whereas the third (Se59) 0.60 ppm for 60 days. Feed and water were provided *ad libitum*. The diet was balanced according to NRC (2007) requirements, with 3.1 Mcal/kg and 10.36 % of crude protein per day. The main ingredients were whole grain sorghum, ground corn, cracker crumbs, rolled corn, DDG (distillers dried grains), bran and molasses. Organic Se was obtained from Se-enriched *Saccharomyces cerevisiae* commercial product (Selyeast 3000; 3000 mg Se/kg). A Se-free mineral mixture was provided for the basal diet. At the beginning of the experiment, sheep were dewormed with ivermectin at a dose of 2 µg/kg of weight and

supplemented with a total dose of 0.5 mL of vitamins A, D and E.

At the end of the experiment, and at average weight of 39.5 ± 4.4 Kg, sheep were slaughtered after fasting for 12 h, at a particular abattoir according to the Official Mexican Standards NOM-033-ZOO-1995.

The pH was recorded immediately after slaughtering and 45 minutes postmortem (pH₄₅) using a potentiometer (Hanna Instruments, model HI 99163, Italy) according to Honikel (1998). The measurements were taken at the 10th rib, then the carcasses were refrigerated at 4 °C for 24 h; time in which were recorded pH (pH₂₄) and temperature. After excision at 24 h postmortem, samples were taken from *Longissimus dorsi* muscle where color was measured directly on the meat surface using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan), after this, each individual sample was then vacuum packaged in clear gas impermeable plastic and transported to laboratory at 4 °C, then packing was removed and the samples were displayed on a flat horizontal surface in a chiller set to 4 °C. The temperature fluctuated between 2 °C and 5 °C.

For shelf life, color was measured with the same Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan), the lightness (L*), redness (a*) and yellowing (b*) values were recorded in triplicate. Hue angle, (H*) = $[\tan^{-1}(b^*/a^*)] * 57.29$ and Chroma, (C*) = $[(a^{*2}+b^{*2})^{1/2}]$ were calculated for each sample, according to Girolami *et al.* (2013) and Ripoll *et al.* (2011). Color and pH were measured at 0, 4, 6 and 8 days of shelf life.

The software Stat Graphics version 5.0 Plus was employed for all statistical tests. MANOVA test was applied for treatments during shelf life. Tukey test was performed to determine significant differences between days during shelf life. Pearson correlations were calculated between pH, L*, a*, b*, C* and H* values. All tests were carried out at 95% confidence level.

RESULTS AND DISCUSSION

Significant interactions were observed between treatment × storage time (Table 1). Color characteristics L*, a*, b*, C* and H* were not differ among treatments (P>0.05- Table 1 and 2). However, sampling time (i.e., 0, 4, 6 and 8 days of the experiments) during shelf life affected (P<0.05)

TABLE 1: MANOVA analyses of the p-values of pH, color and pHx color interaction characteristics during shelf life of meat.

	pH	L*	a*	b*	C*	H*
Treatment (T)	0.6555	0.1727	0.6326	0.8472	0.6016	0.8166
Day (D)	0.0001	0.4190	0.0147	0.0001	0.2158	0.0001
T × D	0.5385	0.8993	0.8597	0.8630	0.8903	0.8643

P-values < 0.05 indicates statistically significant differences

TABLE 2: Effect of organic selenium supplementation on meat pH and color characteristics during shelf life

	Levels of Se*			P	SEM
	Se0	Se34	Se59		
pH					
Day 0	5.63	5.59	5.53	0.6070	0.06
Day 4	6.24	6.24	6.30	0.8749	0.09
Day 6	6.19	6.19	6.01	0.3545	0.09
Day 8	6.03	6.16	6.12	0.1000	0.04
Lightness (L*)					
Day 0	37.07	37.51	37.63	0.9122	0.97
Day 4	37.64	37.17	39.15	0.1190	0.66
Day 6	37.89	37.25	37.82	0.8768	0.95
Day 8	38.08	37.91	39.61	0.3091	0.83
Redness (a*)					
Day 0	14.84	13.48	14.14	0.5941	0.93
Day 4	13.84	14.18	15.66	0.6090	1.34
Day 6	11.82	12.92	13.59	0.6279	1.28
Day 8	11.72	11.99	11.66	0.9615	0.88
Yellowness (b*)					
Day 0	6.18	5.60	6.27	0.6896	0.59
Day 4	8.54	9.12	8.33	0.5116	0.48
Day 6	9.84	9.71	10.23	0.7402	0.49
Day 8	10.35	9.99	10.50	0.8506	0.64
Chroma (C*)					
Day 0	16.10	14.60	15.47	0.6179	1.07
Day 4	16.37	16.90	17.92	0.6282	1.14
Day 6	15.62	16.24	17.06	0.6461	1.07
Day 8	15.72	15.78	15.74	0.9982	0.75
Hue (H*)					
Day 0	21.90	22.43	23.82	0.4634	1.10
Day 4	32.37	32.72	29.47	0.6886	2.88
Day 6	41.02	37.32	37.45	0.6262	3.01
Day 8	41.48	40.03	42.23	0.8612	2.88

*Selenium supplementation expressed as ppm
SEM, Standard error of means

pH, a*, b* and H* (Table 1). The changes during storage time for pH, L*, a*, b*, C* and H* differed between treatments. Both of L* and C* values were not affected during storage time.

Pearson test for the correlations among pH and color characteristics are shown in Table 3. It was found that strong significant positive correlations were found among day and pH, day and b*, day and H*, a* and C*, and b* and H*. The correlation was moderate for pH and b*, pH and H*, and b* and C*. In contrast, it was found that a moderate negative correlations were found among a* and H*, low negative correlations for day and a*, and C* and H*. However, a* value and b* value were not significantly correlated.

No significant differences were observed for pH value after 24 h for all treatment. Values were lower than those reported by Vignola *et al.* (2009) in lambs who obtained a pH 6.21 and 6.18, for 0.3 ppm and 0.45 ppm Se yeast. Komprda *et al.* (2012) reported that a pH for lambs at 24 h

after slaughtering ranged between 5.8-5.74; however, Kuchčík *et al.* (2012) reported a range of 5.63-5.77. In addition, Skrivánová *et al.* (2007) fed calves with diets containing either basal Se or Se-enriched yeast and did not observe any significant influence on meat pH postmortem. In contrast, in finishing pig, Zhan *et al.* (2007) supplemented diets with either sodium selenite or selenomethionine at 0.30 ppm and found increased pH values of loin muscle in Se-treated groups with highest values in animals received selenomethionine. In the current study, the pH found decreased appropriately from 7.0 to a range between 5.3 - 5.8 after slaughtering which is suitable for acceptable meat characteristics by consumers (Devine *et al.*, 1993). Increased pH values reflects the degree of protein breakdown and free amino acids production which cause the formation of NH₃ and amines as well as compounds of alkaline reaction (Stadtman and Levine, 2003; Estévez, 2011).

The Se supplementation did not influenced meat color in the current study, neither 24 h after slaughtered nor

TABLE 3: Pearson correlations between pH and color characteristics for treatments

	pH	L*	a*	b*	C*	H*
Concentration	-0.0331(0.7828)	0.1612(0.1761)	0.0975(0.4151)	0.0163 (0.8919)	0.0931 (0.4366)	-0.0441(0.7127)
Day	0.6084(0.0000)	0.1652(0.1654)	-0.3148(0.0071)	0.7829(0.0000)	0.0625(0.6022)	0.7643(0.0000)
pH		0.1634(0.1701)	0.0330(0.7833)	0.5514(0.0000)	0.2804(0.0170)	0.4250(0.0002)
L*			-0.1990(0.0937)	0.0908(0.4481)	-0.1298(0.2773)	0.1614(0.1755)
a*				-0.1679(0.1585)	0.8759(0.0000)	-0.6497(0.0000)
b*					0.3192(0.0063)	0.8428(0.0000)
C*						-0.2124(0.0733)

P-values < 0.05 indicates statistically significant differences

during storage time at 4 °C. However, there were significant differences in some color meat characteristics during shelf life, because storage time was conditional to oxidative state of meat components. This may depend on oxygen exposure, light and microbiological growth during storage. Several studies on meat samples stored in high oxygen atmosphere showed a negative effect on meat color, indicating its occurrence to protein and lipid oxidation; as a consequence, it generates changes in quality deterioration of meat (Contini *et al.*, 2014; Estévez, 2011).

Lightness (L*) was not affected during shelf life. Vignola *et al.* (2009) reported that neither level of Se supplementation or source (0.30 ppm sodium selenite or 0.30, 0.45 ppm Se yeast) influenced L* color meat; however, Ripoll *et al.* (2011) used 0.3 ppm of sodium selenite and found that L* increased during storage time, which may be due to that they conducted their research until 13 days and reported this change at 7 days while in our study we included only 8 days, and reported an increased L* value without significant difference among days.

The redness (a*) initial value for Se0 was 14.8 and it was lower than that obtained by Vignola *et al.* (2009) for control. In contrasts, Zhan *et al.* (2007) found that the a* value of loin muscle was increased in selenomethionine-treated pigs. In this research, during storage time a* value was decreased for all treatments. It may be due to the oxidation of heme iron from the ferrous state in deoxymyoglobin and oxymyoglobin to the ferric state in metmyoglobin that results in formation of the brownish-red color that consumers find undesirable (Schaefer *et al.*, 1995). In fresh meat sheep, a bright red colour is usually desired (Bekhit and Faustman, 2005).

No significant differences were found for yellowness (b*) values among treatments. These results are consists with Vignola *et al.* (2009) in lambs, Mateo *et al.* (2007) in pigs, Skrivanová *et al.* (2007) in calves for veal production ,with Se-yeast supplementation to the diet at a level of 0.5 ppm. Cai *et al.* (2012) did not find significant differences in meat color of broilers using 0.0, 0.3, 0.5, 1.0, or 2.0 ppm of nano-

Se. Moreover, b* value showed significant differences over time and increased during shelf life for all treatments and this could be indicate that meat turned to the yellow color.

In our study, Se yeast did not affect shelf life and the lack of effect of Se was in agreement with the results observed by Vignola *et al.* (2009) and Ripoll *et al.* (2011), who mentioned that C* was related to the quantity of pigments and high values represent a more vivid color and denote lack of greyness.

Hue angle values did not showed significant differences among treatments. Similarly, Vignola *et al.* (2009) and Ripoll *et al.* (2011) did not detect any color differences for Hue value. In our study, initial hue values for Se yeast treatment Se0 and Se34, were in average 23 and increased quickly with time at days 8 to 30 and up as an indicator of browning or metmyoglobin formation. The Se yeast did not affect color meat but obviously, there was a clear worsening of meat color during storage time.

In relation to Pearson correlations, there were significant positive correlations among day and pH, and b* and H*. This means that when meat is over time, pH increased and become more yellowness and it represents lost meat quality characteristics. This may be due to the more susceptible of meat to the growth of microorganisms when pH increased. The pH had a positive correlations with b*, C* and H*, and this could be due to fact that these oxidation of meat components causes a decreased red meat color with increased yellow color. Moreover a* had a significant positive correlation with C* and negative correlation with H*; it is possible because a* value had a relationship between hemoglobin content and when it lose the saturation of color it change.

CONCLUSIONS

Se-enriched yeast supplementation to the diet of finishing sheep at a level of Se34 ppm or Se59 ppm did not affect meat color or pH characteristics. There are a few researches about effects of Se in meat color, and more researches with higher concentrations of Se yeast and consider more days for storage time are required.

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