PAPER

Effect of organic selenium-enriched yeast supplementation in finishing sheep diet on carcasses microbiological contamination and meat physical characteristics

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Abstract

The aim of the current study was to evaluate the effect of feeding Pelibuey sheep on diet supplemented with different doses of organic selenium (Se)-enriched yeast on carcasses microbiological contamination and meat physical characteristics. The experiment was conducted during the finishing stage of 18 female sheep and lasted for 60 days. In a complete randomized design, sheep were distributed to one of three treatments: the control without Se-yeast (T1), the control supplemented with Se-yeast at 0.35 mg Se/kg DM (T2), and control supplemented with Se-yeast at 0.60 mg Se/kg DM (T3). The yeast product used was Selyeast 3000™ yeast (LFA Lesaffre, Toluca, Mexico) with a Se concentration of 3000 ppm (mg/kg). Lambs were slaughtered at the end of the experiment at an average weight of 39.5±4.41 kg and samples were taken for microbiological analysis. There were no differences between treatments (P>0.05) and the aerobic plate counts for T1, T2 and T3 had indexes of 0.10, 0.08 and 0.08 log_{10} CFU/cm^2, respectively. Total coliform counts obtained were 0.13, 0.10 and 0.09 log_{10} CFU/cm^2 for T1, T2 and T3, respectively, and the faecal coliform counts were 0.09 log_{10} CFU/cm^2 for T1, 0.06 log_{10} CFU/cm^2 for T2 and 0.07 log_{10} CFU/cm^2 for T3. No significant effects (P>0.05) were observed for carcasses physical characteristics of microbial growth, initial and ultimate pH and temperature, colour values and water holding capacity. It can therefore be concluded that organic Se-enriched yeast did not affect carcasses bacterial proliferation or meat physical characteristics.

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Introduction

In recent years, much attention has been paid to meat production with physiological functions that promote health conditions and prevent disease risks. Functional meat value could be increased by adding compounds with antimicrobial and antioxidant functions to the animal's basal diet like phytogenic extracts, conjugated linoleic acid, vitamin E, n-3 fatty acids and selenium (Se) to improve animal production, carcass composition, fresh meat quality and increasing the antioxidant capacity (Grashorn 2007; Zhang *et al.*, 2010; Yanian *et al.*, 2011; Salem *et al.*, 2014a, 2014b). The amount of Se supplementation to diets varies according to the species. In case of sheep, 0.30-0.45 mg/kg DM is the recommended level (Vignola *et al.*, 2009) whether Se supplemented in inorganic or organic forms.

Selenium is an essential trace element for both animal and human health. Selenium is present in tissues and is part of the glutathione peroxidase (GSH-Px) enzyme, which reduces lipid and hydrogen peroxides to less harmful hydroxides via oxidation, and subsequent reduction of selenocysteine and without Se, this enzyme could not act (Juniper *et al.*, 2009; Vignola *et al.*, 2009). Glutathione peroxidases are probably protecting neutrophils from oxygen-derived radicals, which are produced to kill invading organisms (Splettstoesser and Schuff Werner, 2002). Moreover, Se is essential for other cell mediated immunity traits, like removal of viruses and destruction of neoplastic cells (Stazi and Trinti, 2010).

De Vore *et al.* (1983) mentioned that Se antioxidant functions have persisted after slaughter in poultry muscle tissue, via GSH-Px activity. Moreover, Juniper *et al.* (2009) reported that GSH-Px activity was greater in lambs that receiving Se-enriched yeast compared with those receiving a similar dose Se from an inorganic source (sodium selenite). Selenium has the ability to improve immune system as this trace element is essential for the development and expression of non-specific humoral and cell mediated immune responses (Kumar *et al.*, 2009).

The most important factors in fresh meat handling are handling speed, control of temperature and proper hygiene conditions (Ray and Bhunia, 2008). Meat quality factors such as colour and drip loss are decisive for consumer purchase decision. Discoloration of meat is believed to be related to the oxidation processes, and as a consequence sensorial changes and microorganisms proliferation (Baron and Andersen 2002; Wang *et al.*, 2009).

Researches have been done on meat, but there is no information about the effect of organic Se on microbial contamination of carcasses. The hypothesis of the current study was based on the ability of Se to improve immune system, its importance for cell removal of viruses and the destruction of neoplastic cells, which may reduce carcasses microbiological contamination. Therefore, the

aim of this study was to evaluate carcasses microbiological contamination and meat physical characteristics in sheep fed diet supplemented with Se-enriched yeast at different doses.

Materials and methods

Study design

The experiment was conducted during the finishing stage of 18 Pelibuey breed ewes with an initial body weight of 27.75±3.37 kg and final body weight of 39.5±4.41. Animals were randomly assigned to one of three treatments: a control without Se-enriched yeast supplementation (T1), control supplemented with Se-enriched yeast with total Se concentration of 0.35 mg/kg DM (T2) or control supplemented with Se-enriched yeast with total Se concentration of 0.60 mg/kg DM (T3). The yeast product used was Selyeast 3000™ Se-enriched yeast (LFA Lesaffre, Toluca, Mexico), obtained from the growth of *Saccharomyces cerevisiae* on a rich culture medium and fixed intracellularly as seleno-methionine and seleno-cysteine yeast, which makes it a highly bioavailable source of organic Se. Selenium concentration in the product was 3000 ppm (mg/kg). For 60 days, sheep were given a balanced diet according to National Research Council (2007) requirements with an energy concentration of 3.1 Mcal/kg DM and 10.2% of crude protein/kg DM. The diet's main ingredients were: whole grain sorghum, ground corn, cracker crumbs, rolled corn, DDG (distillers dried grains), bran and molasses. Water and feed was offered *ad libitum*, whereas Se-enriched yeast was given individually.

Slaughtering of animals

The sheep were slaughtered in an abattoir in Capulhuac, State of Mexico, Mexico under the Official Mexican Standards NOM-033-ZOO-1995 (Norma Oficial Mexicana, 1995) and NOM-009-ZOO-1994 (Norma Oficial Mexicana, 1994a).

Carcasses sampling

The non-destructive method of the European Commission Directive 2001/471/EC (European Commission, 2011) was used to evaluate the carcass for contamination. After evisceration and before chilling, samples (100 cm^2 per sampling site) were taken from the flank, thorax lateral, brisket, and breast to make a composite sample. The sample surface was delineated by an aluminium sterile template. Sterile swabs with large single-ended cotton wool tip 15 cm long (Protec™, DF, Mexico) were moistened in sterile saline peptone water (Laboratories CONDA, Madrid, Spain) (0.1% peptone + 0.85 % NaCl distilled water) and rubbed vertically, horizontally and diagonally for 20 seconds. Swabs were placed in sterile test tubes (Thomas Scientific, NJ, USA) with 10 mL of sterile saline peptone water. Samples were transported in a cooler (Coleman Company, Inc., Colorado, USA) at 4 °C, and stored at the same temperature until analysing before 24 h.

Microbiological analysis

Test tubes with samples were shaken vigorously for uniform microorganisms distribution. Decimal dilutions of up to 10^{-3} were prepared using test tubes with 9 mL of sterile saline peptone water (0.1 % buffered peptone water, 0.9 % sodium chloride solution) as recommended by NOM-110-SSA1-1994 (Norma Oficial Mexicana, 1994b). Samples were analysed for aerobic plate counts (APC), total coliform counts (TCC) and faecal coliform counts (FCC).

Aerobic plate count

To evaluate the APC, the standard pour plate method as established by Official Mexican Standard NOM-092-SSA1-1994 (Norma Oficial Mexicana, 1994c) was used. All sample dilutions were inoculated in duplicates on to plate count agar (Sigma-Aldrich Co., MO, USA). After solidification plates were incubated at 35±2 °C for 48±1 h.

Total coliform count

The standard pour plate technique was used to quantify total coliform counts (TCC). Violet red bile agar (Sigma-Aldrich Co., MO, USA; VRBA) was poured on to 1 mL of each dilution and when the agar had solidified; approximately 4 mL of RVBA was added. Plates were incubated at 35±2 °C for 24±2 h, according to NOM-113-SSA1-1994 (Norma Oficial Mexicana, 1994d).

Faecal coliform count

Because Mexico does not have an official standard method for pour plate technique, the Association Française de Normalisation (AFNOR) NF V08-60 (1996) method was used. The VRBA was added to each plate with 1 mL of dilution and after solidification a double layer of VRBA was added and the plates incubated at 45±2 °C for 24±2 h.

Physico-chemical characteristics

For the 10th rib, temperature and pH were recorded 45 minutes after slaughtering the sheep (pH_{45}). The carcasses were then refrigerated at 4 °C for 24 h and the pH (pH_{24}) and temperature were recorded again using a potentiometer (Hanna Instruments, model HI 99163, Italy) according to Honikel (1998).

Samples from the *Longissimus dorsi* muscle were taken at 24 h after slaughter to record colour, lightness (L*), redness (a*) and yellowness (b*) using a Minolta Chroma Meter CR-400 (Minolta, Osaka, Japan).

Water holding capacity (WHC) was measured 24 h after slaughter by compression between two petri dishes as described by Cañeque and Sañudo (2005).

Statistical analysis

All bacterial count data were transformed to log_{10} CFU/cm^2 per sample before statistical analysis. Differences between treatments for APC, TCC, FCC, colour, initial and final pH, temperature and WHC were analysed by ANOVA at a significance level of 95% using the statistical package Statgraphics Plus 5.0.

Results and discussion

Microbiological profile

The microbiological variables APC, TCC and FCC were not different among treatments (P>0.05). However, the APC in carcasses of sheep supplemented with 0.60 mg/kg of Se was numerically lower (P>0.05) by about 20 %. The total coliform loads in T3 were numerically lower by about 30.2%, while the faecal coliforms counts were numerically lower by about 30.1% (Table 1). Aerobic plate count is a very widely used test to estimate general contamination and is accepted as a criterion for carcasses surface microbial contamination. However, *Enterobacteriaceae* (*E. coli*, *Salmonella* spp., *Serratia liquefaciens, Pantoea agglomerans, Klebsiella pneumonia, Enterobacter cloacae*) counts are indicators of faecal contamination, and in combination, the two determinations are used as a criterion for the verification of slaughter hygiene (Zweifel and Stephan, 2003; Hauge *et al.*, 2011).

The European Commission Directive 2001/471/EC uses the total viable count (TVC) and *Enterobacteriaceae* as bacterial indicators of hygiene and faecal contamination on carcasses before chilling (Lenahan *et al.*, 2010). In the current study, the mean values of TVC were within acceptable range according to the EC Commission Directive 2001/471/EC of < 3.5 log_{10} CFU/cm^2. Treatments T1, T2 and T3 had TVC indexes of 0.10, 0.08 and 0.08 log_{10} CFU/cm^2, respectively. Our values are lower than those reported by Sumner *et al.* (2003)

from South Australia abattoirs with 2.8 $\log_{10}$ CFU/cm^2. Moreover, Zweifel and Stephan (2003) in Swiss abattoirs, and Salmela *et al.* (2013) in Finland abattoirs studied the microbiological contamination of sheep carcasses and reported APC mean values of 2.5 and 3.16 $\log_{10}$ CFU/cm^2, respectively for the carcasses. All these results were in accordance with EC Commission Directive 2001/471/EC. However, Bhandare *et al.* (2007) and Hauge *et al.* (2011) reported a mean APC of 4.82 to 6.06 $\log_{10}$ CFU/cm^2 with sheep and goat which are higher than those acceptable according to EC Commission Directive 2001/471/EC.

Total coliform count values of 0.13, 0.10 and 0.09 $\log_{10}$ CFU/cm^2 were obtained for treatments, T1, T2 and T3, respectively. To our knowledge, there are no studies on sheep carcasses to compare these total coliforms counts to therefore, the results were compared to those of other animal species. These results are comparable with those of San Juan *et al.* (2007) and Nouichi and Hamdi (2009) who obtained value of TCC of 1.03 $\log_{10}$ CFU/cm^2 and 2.92 $\log_{10}$ CFU/cm^2, respectively, in bovine carcasses in a slaughterhouse in Algeria.

Our results of faecal coliform count are lower than the cutoff recommended by the EC Commission Regulation (European Commission, 2001). Other studies have shown higher FCC values (Bhandare *et al.*, 2007; Nouichi and Hamdi, 2009) with mean values of 2.55 to 3.50 $\log_{10}$ CFU/cm^2 for ovine carcasses in Algerian and Indian slaughterhouse, respectively.

The activity of organoselenium compounds against microorganisms was evaluated by Pietka-Ottlik *et al.* (2008) who showed no activity with Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus simulans*), whereas for Gram-negative bacteria (*Escherichia coli, Pseudomonas aeuginosa, Klebsiella pneumonia*) was substantially lower.

Based on the above, it can be suggested that Se reduced the bacterial count. However, Se antimicrobial activity is not completely understood. ALQuthami *et al*. (2014) studied the antibacterial effect of Se and obtained cell disintegration because of cytoplasmic constituents leakage and cell dehydration. Yang *et al.* (2009) evaluated Se-enriched probiotics' antibacterial action *in vitro* and *in vivo* in mice and reported a strongly antagonize pathogenic of *Escherichia coli* in both *in vitro* and *in vivo*.

Physical characteristics

Carcasses physical characteristics of microbial growth, initial and final pH, temperature, colour values (L*, a* and b*) and WHC are presented in Table 2. There were no differences (P>0.05) among treatment in initial and final pH, temperature at 45 min after slaughter and after 24 h of chilling, L*, a* and b*. However, differences were observed among treatments for WHC. Treatment of T_2 had lower (P<0.05) WHC at 0.35 µg/kg Se compared to other treatments (Table 2). There were no differences (P>0.05) in initial and final pH and temperature. These findings are in agreement with Vignola *et al.* (2009) who also did not find any difference between treatments with different Se sources and levels. In contrast, Li *et al.* (2011) found that pH was lower in pigs fed Se free diet. In general, the muscle pH of living animals is normally around 7.4, but after death, the pH falls to 5.5 – 5.8 as a result of muscles glucose converting into lactic acid (Corry, 2007). Therefore, our results of pH falling from 7.15 to 5.53 is consistent.

The pH value has effects on colour, shelf life, taste, microbiological stability, yield and texture of the meat. At a pH of 6.4, meat is tainted due to enzyme activity, thus producing large amounts of metabolic by-products, foul smell, sliminess and discolouration (Feiner, 2006). Bacterial proteolytic enzymes operate best near neutral pH, and the enzymes which attack carbohydrates tend to have an optimal pH below 6. Organisms such as lactic acid bacteria whose predominant activity is carbohydrate breakdown, have an optimal pH between pH 5.5 and 6.0 (Lawrie and Ledward, 2006). The final pH in the present study was in the range of 5.3 to 5.8 which is near to the optimal microbial growth pH.

Cherry red colour (a*) is one of the most important qualities of meat for consumer purchase decision. It is an indicator of freshness

Table 1. Microbiological profile (mean±standard deviation) of carcasses from sheep fed diets supplemented with selenium-enriched yeast.

Items	T1	T2	T3	P value
Aerobic plate count, $\log_{10}$ CFU/cm^2	0.10±0.09	0.08±0.05	0.08±0.01	0.881
Total coliforms count, $\log_{10}$ CFU/cm^2	0.13±0.06	0.10±0.06	0.09±0.07	0.590
Faecal coliforms count, $\log_{10}$ CFU/cm^2	0.09±0.06	0.06±0.02	0.07±0.08	0.812

Diets contained energy concentration of 3.1 Mcal/kg DM and 10.16% of crude protein/kg DM without Se-enriched yeast supplementation (T1), or supplemented with Se at 0.35 mg/kg DM (T2) or supplemented with Se at 0.60 mg/kg DM (T3).

Table 2. Physical characteristics (mean±standard deviation) of meat of sheep fed diets supplemented with selenium-enriched yeast.

Variable	T1	T2	T3	P value
pH_{45}	7.06±0.24	7.03±0.36	7.15±0.52	0.857
pH_{24}	5.63±0.15	5.59±0.19	5.53±0.13	0.607
Temperature 45 min, °C	20.98±2.95	21.15±3.13	26.6±3.24	0.952
Temperature 24 h, °C	13.52±1.05	12.78±1.46	12.95±1.50	0.626
L*	37.07±2.67	37.51±2.62	37.63±1.69	0.912
a*	14.84±3.13	13.48±1.75	14.14±1.61	0.594
b*	6.18±2.07	5.59±0.95	6.27±1.08	0.690
WHC (% juice released)	11.65±2.39^a	8.62±2.67^b	11.96±2.73a	0.039

a*, redness; b*, yellowness; L*, lightness; WHC, water holding capacity. Diets contained energy concentration of 3.1 Mcal/kg DM and 10.16% of crude protein/kg DM without Se-enriched yeast supplementation (T1), or supplemented with Se at 0.35 mg/kg DM (T2) or supplemented with Se at 0.60 mg/kg DM (T3). ^{a,b}Means within in the same row with different superscripts differ significantly among treatments (P<0.05).

and quality (Brewer *et al.*, 2001; Mancini and Hunt, 2005). In the current study, there were no differences between treatments for colour values of a*, b* and L*. However, Vignola *et al.* (2009) in lambs, found higher values for L*, a* and b* where values were 44.63, 15.44 and 6.76, respectively. In pigs, Li *et al.* (2011) reported that Se did not had effect on meat colour values of a*, b* and L*. Preventing ferrous myoglobin from oxidation is a critical factor for maintaining meat colour stability. A high level of GSH in meat tissues is associated with a high reducing capacity, reducing the formation of H_2O_2, and soon afterward oxidation of ferrous iron at the same time causing maintain meat colour stability (Zhan *et al.*, 2007; Liu *et al.*, 2011). They reported that selenomethionine- treatments increased redness of meat.

In the current study, the treatment 0.35 mg T_2 presented a lower percentage of juice released, therefore higher WHC. Zhan *et al.* (2007) evaluated the effect of different Se source added at 0.30 mg Se/kg to basal diet on loin meat quality in finishing pigs and reported values of 14.3, 14.0 and 12.5% for the control, sodium selenite treatment and selenomethionine-treated groups, respectively after 16 h exposure in a 25 °C room with significantly lower drip loss with the selenomethionine-treated group. Wang *et al.* (2009) and Li *et al.* (2011) mentioned that drip loss of meat decreased with the increase of dietary Se level in poultry and pigs. Generally, Se as part of GHS- Px elevates and maintains this enzyme activity, protect cell membranes from oxidation and improving meat WHC (Mateo *et al.*, 2007; Wang *et al.*, 2009).

Conclusions

Although the differences were not significant, sheep supplemented with 0.60 µg/kg Se in the diet had a 20% lower aerobic plate counts in the carcasses, 30% lower total coliform count and a 30% lower faecal coliforms count than un-supplemented sheep. Drip loss was lower for sheep fed the 0.35 mg/kg DM dose. From these results we can conclude that organic Se-enriched yeast did not affect carcasses bacterial proliferation or meat physical characteristics. More studies with larger numbers of animal are recommended to study the effect of organic Se supplementation on carcasses microbiological contamination and meat physical characteristics.

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