

# Effect of supplementation with vitamin E and chelated or inorganic minerals on beef quality and oxidative stability



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#### **Abstract:**

Diet and supplementation during finishing beef cattle affect meat properties. An evaluation was done of the effects of chelated and inorganic minerals (Cu, Se and Zn), in combination with vitamin E, on beef quality and oxidative stability. A total of 799 zebu x European cattle were used at a commercial feedlot-finishing center in the state of Veracruz, Mexico. Four experimental diets were formulated based on a standard high-grain finishing diet and supplementation with identical doses of Cu, Se and Zn and vitamin E: chelated minerals only; chelated minerals + vit E; inorganic minerals only;

inorganic minerals + vit E. These were fed to the animals for thirty (30) days prior to slaughter. Weight at slaughter was  $450.5 \pm 30.5$  kg. Twelve individuals were randomly selected from each treatment to evaluate quality variables in the *Longissimus thoracis* muscle. Meat samples were stored at -20 ° C until processing. Samples were defrosted and aged at 4 °C for one and eight days. Water loss from defrosting was lowest in the inorganic minerals treatments (P<0.05). In the chelated minerals treatments, pH, water holding capacity and catalase activity were higher (P<0.05), and shear force was lower (P<0.05). Vitamin E decreased drip water loss (P<0.05). After eight days' aging, use of inorganic minerals without vitamin E allowed greater oxidative activity, as shown by the thiobarbituric acid-reactive substances values. The combination of chelated minerals and vitamin E resulted in lower water loss, oxidative activities and cutting force, and is recommended for use in finishing diets for beef cattle.

**Key words:** Meat quality, Oxidative stability, Beef, Chelated minerals, Inorganic minerals.

Received: 06/04/2018

Accepted: 11/03/2019

# Introduction

Supplementing the feed of growing animals with chelated minerals and vitamin E is reported to improve meat physicochemical and organoleptic characteristics<sup>(1)</sup>. This kind of supplementation has been found to increase meat quality and degree of marbling<sup>(2)</sup>, increase beef hot carcass weight<sup>(3,4)</sup>, decrease water loss from drip in carcasses<sup>(5)</sup>, and improve color in pork<sup>(6)</sup>. Vitamin E supplementation in beef cattle diets has been found to lower lipid oxidation<sup>(7)</sup>, and oxymyoglobin oxidation<sup>(8)</sup>.

Chelated minerals are more efficiently absorbed<sup>(9)</sup> and more available<sup>(10)</sup> in animals, which improves their distribution and retention in tissues<sup>(7,11)</sup>. In contrast, inorganic minerals can dissociate in the reticulum-rumen, omasum and abomasum, forming indigestible compounds<sup>(12)</sup> and insoluble complexes with other minerals<sup>(13)</sup>. Copper (Cu), selenium (Se) and zinc (Zn) are essential minerals<sup>(14)</sup>. They are cofactors of antioxidant enzymes such as glutathione peroxidase (GPX)<sup>(15,16)</sup> and superoxide dismutase (SOD)<sup>(17)</sup>, and are involved in protecting the cytoplasmic membrane from oxidative damage.

Another micronutrient that has a meat-enhancing and preservative effect is vitamin E, a natural antioxidant located in the cell membrane which protects fatty acids from oxidation<sup>(18)</sup>.

The present study objective was to analyze quality and oxidative stability in meat from feedlot-finished beef cattle in the tropics of Mexico fed a high-grain diet supplemented with Cu, Se, and Zn from chelated or inorganic sources, and with or without vitamin E.

#### Material and methods

The study was carried out at a beef cattle finishing commercial unit corral in the southern portion of the state of Veracruz, Mexico (19°38'00" N; 95°31'00" W). Experimental animals were 799 beef cattle (713 females and 86 males; Bos taurus x Bos indicus) obtained from stockers in states throughout southern Mexico (Veracruz, Oaxaca, Tabasco and Chiapas). Average initial weight when placed in finishing pens was 315.9 + 4.52 kg. Animals were randomly assigned to house in 16 pens per treatment. Thirty (30) days before slaughter the animals were fed a finishing diet including a basic inorganic mineral base premix (Table 1). One of two Cu/Se/Zn premixes was added to the diets, with the same amount of minerals regardless of their source (Table 2): chelated minerals (Bioplex® Copper, Bioplex® Zinc and SelPlex®, Alltech Mexico); or inorganic minerals (zinc oxide, copper sulfate and sodium selenite). Each animal consumed an approximate daily dose of 4 g mineral premix containing 93.9 g/kg Zn, 25 g/kg Cu and 0.757 g/kg Se. Vitamin E (DSM, Mexico) was supplemented at 1320 UI/head/day. Both the minerals and vitamin E were administered following a 2 x 2 treatment factorial arrangement: T1) finishing diet plus inorganic minerals; T2) T1 plus vitamin E; T3) finishing diet plus chelated minerals; T4) T3 plus vitamin E. The experimental diets were provided twice a day (40% at 0600 h; 60% at 1200 h). Animals had free access to water.

**Table 1:** Calculated composition and nutritional value of the diet fed to beef cattle for the last 30 days finishing period in feedlot

Ingredients	%	Nutritional value:		
Rolled corn	76.7	Dry matter	87.71 %	
Wheat bran	5.0	Net metabolizable energy	2.34 Mcal/kg	
Barley hay	4.5	Crude protein	12.88 %	
Molasses	4.0	Ether extract	7.32 %	
Soybean meal	4.0	Ashes	4.56 %	
Soybean oil	3.3	Neutral detergent fiber	12.67 %	
Inorganic mineral premix <sup>1</sup>	2.5	Net energy for gain	1.65 Mcal/kg	
		Metabolizable energy	3.31 Mcal/kg	
		Digestible energy	3.97 Mcal/kg	
		Rumen degradable protein	60.46 %	

<sup>&</sup>lt;sup>1</sup> Calcium (5.75 g/kg), magnesium (2.35 g/kg), copper (16.42 mg/kg), selenium (0.06 mg/kg), zinc (43.41 mg/kg).

**Table 2:** Composition of experimental mineral premixes fed beef cattle for the last 30-day finishing period in feedlot

<b>Chelated mineral</b>	premix	Inorganic mineral premix			
Ingredients:	(mg of mineral/kg)	Ingredients:	(mg of mineral /kg)		
Zinc proteinate	93900	Zinc oxide	93900		
Copper proteinate	25000	Copper sulfate	25000		
Selenium yeast	757	Sodium selenite	757		

Packaging: mineral premixes were prepared in 25-kilogram lots.

Dose: 4 g/head/d.

# Cattle transport and slaughter

Using specialized vehicles, the cattle were transported 110 km from the growing area to a Federal Inspection Type (Tipo Inspección Federal - TIF) slaughterhouse. Twelve animals per treatment (three per pen) were randomly selected for meat quality and oxidative stability measurements. Using a scale (Revuelta RGI model), the animals were weighed individually upon arriving at the slaughterhouse; average weight for males and females was  $450.5 \pm 30.5$  kg. Transport and slaughter of animals complied with applicable federal regulations: NOM-051-ZOO-1995<sup>(19)</sup>, and NOM-033-ZOO-1995<sup>(20)</sup>. The carcasses were electrically stimulated with alternating current (60 Hz, 50

volts x 2 min) immediately after slaughter, with electrodes placed on the Achilles tendon and the nose.

# pH measurement and meat samples

The carcasses were cut longitudinally to produce two half carcasses and placed in cold storage (0.3 °C). Measurement of pH was done 45 min postmortem in the semimembranosus muscle using an electrode connected to a potentiometer (Hanna Instruments®). A meat sample (approx. 15 cm long) was taken from the *Longissimus thoracis* muscle between the fifth and thirteenth intercostal spaces of the left half of each carcass for meat quality analysis. The carcasses remained in refrigeration for  $24 \pm 2 \text{ h}$ .

# Color and pH measurements in fresh meat

Color and pH measurements were done of meat samples from each carcass 24 h postmortem. After 30 min blooming, color was measured in triplicate with a spectrophotocolorimeter (MiniScan EZ HunterLab®, USA. Iluminante D65/10°) and recorded as L\* (luminosity), a\* (red to green tones) and b\* (yellow to blue tones). Measurement of pH was done with a previously calibrated portable digital potentiometer (pH-meter, Hanna Instruments®, USA). All analyses were done in triplicate<sup>(21)</sup>. After the measurements were taken the samples were vacuum packed and frozen at -20 °C until analysis at the Meat Laboratory of the National Animal Physiology and Improvement Disciplinary Research Center (Centro Nacional de Investigación Disciplinaria en Fisiología y Mejoramiento Animal) of the INIFAP in Colón, Querétaro, México.

#### Sample preparation and analysis of aged meat

Using a bench saw (St-295-PE, Torrey®, Mexico), each frozen sample was sectioned into five approximately 1-inch-thick cutlets from the region near the cranial area. The cutlets were weighed and numbered as cut, and placed in a vertical refrigerator (Torrey®, Mexico) at 2 °C. Once defrosted they were weighed again to calculate water loss by subtracting defrosted weight from frozen (-20 °C) weight, which was expressed as the percentage weight lost compared to the initial weight. Cutlet No. 1 from each individual was used to evaluate pH<sup>(22)</sup>, color<sup>(23,24)</sup>, water holding capacity<sup>(25)</sup>, concentration of thiobarbituric acid-reactive substances (TBARS)<sup>(26)</sup>, and activities of the enzymes glutathione peroxidase (GPX) and catalase (CAT)<sup>(27)</sup>. Cutlet No. 3 was used to measure drip water loss<sup>(28)</sup>. Cutlet No. 4 was prepared on an electric grill and then measurements were taken of shear force and water loss from cooking<sup>(29)</sup>. Cutlets numbers 2 and 5 were placed on foam trays, covered with plastic wrap and aged for 8 days at 2 °C. After aging, measurements were taken of pH, color, water loss from cooking, shear force, lipid oxidation by TBARS, GPX and CAT activity. All measurements were done in triplicate.

#### Statistical analysis

All variables were processed with an ANOVA using a 2 x 2 factorial arrangement, and PROC MIXED in the SAS ver. 9.3 package.

Statistical model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + E_{ijk}$$

 $\mu$  = effect of general mean;

 $\alpha_i$  = effect of i-th treatment of mineral source;

 $\beta_j$  = effect of j-th treatment of vitamin E;

 $(\alpha\beta)_{ij}$  = effect of mineral source / vitamin E interaction;

 $E_{ijk}$  = random error of each observation.

Also using SAS, a multivariate analysis with Pearson correlations was run to evaluate the relationships between the studied variables.

#### **Results and discussion**

# Muscle pH values 45 min postmortem

At 45 min postmortem, no differences (P>0.05) in pH were observed due to mineral source, vitamin E or their interaction (Table 3). Values ranged from 6.1 to 6.3, which are similar to those reported in studies using electrical stimulation after bleeding in cattle<sup>(30)</sup>.

**Table 3:** Response of pH and color to supplementation with Se, Cu and Zn from inorganic or chelated sources, with or without vitamin E in meat from feedlot -finished cattle in the tropics

	Mineral	l source	Vitamin E			
Variable	Inorganic (n= 24)	Chelated (n= 24)	No (n= 24)	Yes (n= 24)		
pН						
45 min postmortem	6.31 <u>+</u> 0.05	6.23 <u>+</u> 0.07	6.21 <u>+</u> 0.05	6.33 <u>+</u> 0.07		
24 h postmortem	5.55 <u>+</u> 0.03 <sup>a</sup>	5.67 <u>+</u> 0.05 <sup>b</sup>	5.64 <u>+</u> 0.06	5.59 <u>+</u> 0.02		
L* (luminosity)						
24 h postmortem	41.28 <u>+</u> 0.55	41.74 <u>+</u> 0.70	41.63 <u>+</u> 0.74	41.38 <u>+</u> 0.51		
a* (red tone)						
24 h postmortem	19.14 <u>+</u> 0.53	19.52 <u>+</u> 0.35	19.13 <u>+</u> 0.49	19.53 <u>+</u> 0.41		
b* (yellow tone)						
24 h postmortem	16.95 <u>+</u> 0.41 <sup>a</sup>	17.63 <u>+</u> 0.40 <sup>b</sup>	17.12 <u>+</u> 0.49	17.46 <u>+</u> 0.30		

Results are presented as least squares means  $\pm$  standard error and the n value.

ab Different letter superscripts in the same row indicate significant difference (P<0.05) due to mineral source.

# pH values 24 h postmortem, and after 1 and 8 days' aging in laboratory

After 24 h refrigeration meat pH differed by mineral source (P<0.05), but no effect was found for vitamin E or the mineral source / vitamin E interaction. In the chelated minerals treatment pH values were higher (5.67 ± 0.05) than in the inorganic minerals treatment (5.55 ± 0.03; Table 3). This same effect on pH has been reported in response to supplementation with Se from chelated and inorganic sources<sup>(11)</sup>, although always within ranges considered normal (5.4 to 5.87)<sup>(31,32)</sup>, that do not affect meat organoleptic characteristics. However, these small variations in pH may have affected the configuration of some proteins, which in turn may be related to the observed differences in meat water holding capacity after defrosting, since pH was more acidic in the inorganic source treatment than in the chelated source treatment. No differences in pH were observed between the one and eight days' aging treatments.

#### Color, water holding capacity and oxidative stability after aging

Oxidation of polyunsaturated fats in beef rapidly causes rancidity, but also affects its color, quality and texture<sup>(34)</sup>. In terms of color, luminosity (L\*) was not affected (P>0.05) by mineral source, vitamin E, their interaction or aging period (Table 3 and 4). In contrast, a\* and b\* values were affected (P<0.05) by aging period in all treatments. Red and yellow tones decreased in the meat samples as aging period increased (Table 5), which is associated with the negative correlation between the a\* value and TBARS<sup>(33)</sup>, and the related red tones with oxidation processes.

**Table 4:** Meat quality variables in defrosted meat from feedlot-finished cattle in the tropics after 1 and 8 days' aging, in response to supplementation with Se, Cu and Zn from inorganic or chelated sources, with or without vitamin E

	Minera	l source	Vitamin E		
Variable	Inorganic	Chelated	No (n. 24)	Yes	
	(n=24)	(n=24)	(n=24)	(n=24)	
Water loss from defrosting,%	$2.67 \pm 0.024^{a}$	$3.96 \pm 0.29^{b}$	2.98 <u>+</u> 0.25	3.64 <u>+</u> 0.33	
Water loss from drip, %	7.65 <u>+</u> 0.47	8.33 <u>+</u> 0.40	$8.36 \pm 0.44^{\beta}$	$7.62 \pm 0.44^{\theta}$	
Water holding capacity, %	$6.23 \pm 0.65^{a}$	$9.44 \pm 0.90^{b}$	7.94 <u>+</u> 0.84	7.73 <u>+</u> 0.86	
рН					
1 day's aging	5.52 <u>+</u> 0.01	5.57 <u>+</u> 0.04	5.57 <u>+</u> 0.04	5.51 <u>+</u> 0.01	
8 days' aging	5.61 <u>+</u> 0.01	5.63 <u>+</u> 0.05	5.64 <u>+</u> 0.05	5.59 <u>+</u> 0.01	
L* (luminosity)					
1 day's aging	41.65 <u>+</u> 0.66	41.16 <u>+</u> 0.46	41.52 <u>+</u> 0.66	41.29 <u>+</u> 0.46	
8 days' aging	41.77 <u>+</u> 0.62	42.65 <u>+</u> 0.65	41.79 <u>+</u> 0.73	42.63 <u>+</u> 0.52	
a* (red tone)					
1 day's aging	$18.12 \pm 0.22^{1}$	$17.85 \pm 0.33^{1}$	$18.08 \pm 0.27^{1}$	$17.89 \pm 0.29^{1}$	
8 days' aging	$16.73 \pm 0.38^2$	$16.44 \pm 0.37^2$	$16.47 \pm 0.37^2$	$16.70 \pm 0.38^2$	
b* (yellow tone)					
1 day's aging	16.68 <u>+</u> 0.31 <sup>a</sup>	15.88 <u>+</u> 0.32 <sup>b</sup>	16.26 <u>+</u> 0.37	16.30 <u>+</u> 0.28	
8 days' aging	16.32 <u>+</u> 0.37 <sup>a</sup>	15.61 <u>+</u> 0.24 <sup>b</sup>	16.05 <u>+</u> 0.34	15.88 <u>+</u> 0.30	
Shear force, kg					
1 day's aging	$6.35 \pm 0.28^{1}$	$6.14 \pm 0.28^{1}$	$6.07 \pm 0.26^{1}$	$6.42 \pm 0.30^{1}$	
8 days' aging	$4.70 \pm 0.18^{a2}$	$3.57 \pm 0.12^{2b}$	$4.06 \pm 0.19^2$	$4.18 \pm 0.20^2$	
Water loss from cooking, %					
1 day's aging	$24.44 \pm 0.65^{1}$	$25.40 \pm 0.68^{1}$	$25.22 \pm 0.70^{1}$	$24.62 \pm 0.64^{1}$	
8 days' aging	$22.77 \pm 0.68^2$	$22.64 \pm 0.59^2$	$22.31 \pm 0.53^2$	$23.08 \pm 0.71^2$	

Results are presented as least means squares  $\pm$  individual standard error and the n value. <sup>ab</sup> Different letter superscripts in the same row indicate significant difference (P < 0.05) due to mineral source. <sup>1,2</sup> Different numerical superscripts between rows indicate a significant effect of day. <sup> $\beta$ ,0</sup> Greek letter superscripts indicate an effect from vitamin E.

**Table 5:** Effect of Se, Cu and Zn supplementation from inorganic and chelated sources with and without vitamin E on the oxidative stability of the backs of bovine cattle ended in a pen in the tropics

	WLD	WHC	WLF	WLC	nU	L*	a*	b*	SF	TBARS	CAT	CDV
	WLD	WITC	VVLF	WLC	рН		а 	Ŋ	JF	IDAKS	CAI	GFA
WLD	1.00											
WHC	0.12	1.00										
WLF	0.33*	0.03	1.00									
WLC	-0.04	0.03	0.00	1.00								
рН	-0.28**	0.14	-0.26	-0.29**	1.00							
L*	0.18	0.04	-0.29*	-0.03	-0.42***	1.00						
a*	-0.31**	-0.16	-0.01	0.05	-0.18*	0.05	1.00					
b*	-0.02	-0.18	-0.22	0.02	-0.45***	0.66***	0.68***	1.00				
SF	-0.16	0.25	0.04	0.53***	-0.23*	-0.13	0.19	0.07	1.00			
TBARS	0.26**	0.08	0.40**	-0.17	0.06	-0.04	-0.45***	-0.09	-0.37***	1.00		
CAT	0.07	0.00	0.18	-0.04	0.09	-0.14	0.16	-0.16	-0.24*	-0.09	1.00	
GPX	0.35***	0.20	0.16	-0.32**	0.22*	0.03	-0.38***	-0.15	-0.60***	-0.59***	0.00	1.00

WLD= Water loss from drip; WHC= Water holding capacity; WLF= Water loss from defrosting; WLC= Water loss from cooking; SF = shear force; TBARS= thiobarbituric acid-reactive substances; CAT= catalase, GPX= glutathione peroxidase.

\*P<0.05: \*\*P<0.01: \*\*\*P<0.001.

The value of b \* was higher in treatments with inorganic minerals (P<0.05) at one and eight days' aging (Table 4). This difference was due to an increase in oxidation of oxymyoglobin to metmyoglobin<sup>(35)</sup>, which produced a brown color<sup>(34)</sup>. Greater TBARS activity at eight days' aging in the inorganic mineral treatments was largely responsible for this phenomenon (Table 6).

**Table 6:** Oxidative stability variables in defrosted meat from feedlot-finished cattle in the tropics after one or eight days' aging in response to supplementation with Se, Cu and Zn from inorganic or chelated sources, with or without vitamin E

	Inorganic n	nineral source	<b>Chelated mineral source</b>			
	No vit E	Vit E	No vit E	Vit E		
Variable	T1	T2	T3	T4		
v uriubic	(n=12)	(n=12)	(n=12)	(n=12)		
$TBARS^{\alpha}$	_			_		
(mg MDA/ kg meat)						
1 day's aging	$0.05 \pm 0.008^{\ 1}$	$0.03 \pm 0.003^{\ 1}$	$0.05 \pm 0.006^{\ 1}$	$0.05 \pm 0.004^{-1}$		
8 days' aging	0.72 <u>+</u> 0.107 <sup>2a</sup>	0.24 <u>+</u> 0.017 <sup>2 b †</sup>	0.33 <u>+</u> 0.068 <sup>2 b</sup>	0.20 <u>+</u> 0.021 <sup>2</sup> b † **		
CAT <sup>β</sup> (U/ml extract)						
1 day's aging	10.83 <u>+</u> 0.89 ab	8.32 <u>+</u> 1.08 <sup>a</sup>	12.64 <u>+</u> 0.69 <sup>b</sup>	12.92 <u>+</u> 0.94 <sup>b</sup>		
8 days' aging	9.72 <u>+</u> 1.07 <sup>a</sup>	10.72 <u>+</u> 1.21 <sup>a</sup>	12.62 <u>+</u> 0.83 <sup>b</sup>	13.16 <u>+</u> 0.69 <sup>b</sup>		

$GPX^{\gamma}$ (U/g meat)				
1 day's aging	13.29 <u>+</u> 1.00 <sup>1</sup>	13.99 <u>+</u> 0.77 <sup>1</sup>	15.46 <u>+</u> 0.84 <sup>1</sup>	15.34 <u>+</u> 0.84 <sup>1</sup>
8 days' aging	59.44+4.39 <sup>2</sup>	57.83 <u>+</u> 3.56 <sup>2</sup>	49.43 <u>+</u> 2.80 <sup>2</sup>	51.31 <u>+</u> 3.88 <sup>2</sup>

<sup>α</sup> Thiobarbituric acid-reactive substances; <sup>β</sup> Catalase; <sup>γ</sup> Glutathione peroxidase. Results are presented as least square means  $\pm$  individual standard error and the n value. <sup>ab</sup> Different letter superscripts in the same row indicate significant difference (P<0.05) due to mineral source. The <sup>†</sup> superscript indicates an effect from vitamin E. The \*\* indicates a significant effect from the mineral source / vitamin E interaction. <sup>1,2</sup> Different numerical superscripts between rows indicate a significant effect of aging time.

#### Water loss from defrosting

Mineral source affected (P<0.05) water loss from defrosting (WLF), but vitamin E and the mineral source / vitamin E interaction did not. Water loss was greater (P<0.05) in cutlets in the chelated minerals treatment than in the inorganic minerals treatment. At 24 h postmortem cutlet pH was higher in the chelated than in the inorganic minerals treatment (P<0.05), which favored stability of myofibrillar proteins and therefore water retention. A portion of the water retained in the meat in the chelated minerals treatment may have become ice crystals when frozen; when defrosted these crystals would have caused greater water loss during aging. In addition, the higher CAT activity in this treatment may be related to this greater water loss.

# Water loss from drip

Vitamin E in the diets affected water loss from drip (WLD) (P<0.05), although mineral source and the mineral source / vitamin E interaction did not (P>0.05, Table 4). Supplementation with vitamin E favored antioxidant activity at the cell membrane level<sup>(36)</sup> and allowed the cell to preserve its sarcoplasmic components during storage<sup>(37)</sup>. This antioxidant action is also linked to the lower TBARS activity in the vitamin E-supplemented treatments (Table 5).

# Water holding capacity

Water holding capacity (WHC) was higher (P<0.05) in the chelated mineral treatments than in the inorganic mineral treatments. Vitamin E and the mineral source / vitamin E interaction had no effect (P>0.05). One of the conditions that can alter the arrangement of myofibrillar proteins and the space between them is net charge. This can be modified by changes in the anion/cation balance, especially the replacement of bivalents with monovalents<sup>(38)</sup>, as in the case of added saline solution. In contrast, carcasses and cutlets from the inorganic mineral source treatments exhibited less water loss, even when showed less retention to the added saline solution used for the water holding capacity test. At 24 h postmortem the pH values in the chelated minerals treatments (Table 3) favored WHC because the myofibrillar proteins were further from their isoelectric point (pH 5.4-5.5)<sup>(38)</sup>, leading to protein stability and their binding to water molecules.

#### Shear force after 1 and 8 days' aging

After one day of aging shear force (SF) was unaffected (*P*>0.05) by mineral source, vitamin E or their interaction. However, at eight days SF had decreased (*P*<0.05) in response to mineral source, but not due to vitamin E or the mineral source / vitamin E interaction (Table 4). Shear force positively correlated to water loss from cooking (r= 0.53; *P*<0.001; Table 6) and negatively correlated to pH (r= -0.23, *P*<0.05). A scale developed by Shackelford *et al*<sup>(39)</sup> for SF in beef uses four categories: <3.2 kg= very soft meat; 3.2 to 3.89 kg= soft meat; 3.89 to 4.59 kg= intermediate; and >4.6 kg= hard. Based on this scale, after one day aging the cutlets in all four treatments would be considered "hard". By day eight the cutlets in the chelated minerals treatment qualified as "soft", those in the inorganic minerals only treatment were "intermediate", and those in the inorganic minerals / vitamin E treatment were "hard".

Values for pH equal to or greater than 5.8 are considered unacceptable in beef, and produce dark-colored cuts<sup>(40)</sup>. These are characterized by having higher WHC and lower SF<sup>(41,42)</sup>, as well as high pH values (>6.0) which can increase Z disk degradation<sup>(43)</sup>. The cutlets from the chelated mineral treatments exhibited higher pH values, and thus also had greater WHC and lower SF values. Another factor that may have lowered SF is supplementation with chelated Se, which causes greater accumulation of seleno amino acids, and modifies muscle tissue structure in such a way that it lowers SF<sup>(11)</sup>. Softening

of meat is related to aging during which enzymes such as calpain and cathepsin exercise proteolytic action on muscle fiber structural proteins<sup>(38,44)</sup>. After 8 days' aging enzymatic action had noticeably softened the cutlets, which was particularly favored by higher pH values in the chelated minerals treatments (Table 3).

# Water loss from cooking after 1 and 8 days' aging

Water loss from cooking of cutlets after one or eight days' aging was not affected by mineral source, vitamin E, or their interaction. Aging time did have an effect since water loss was higher after one day than at eight days (Table 4). All the cutlets subjected to cooking had been previously frozen. This can modify WHC<sup>(45)</sup> by causing immobilized water to become ice crystals<sup>(44)</sup>, which, when defrosted, become free water that can be lost during the aging process.

# TBARS in cuts after one and eight days' aging

Thiobarbituric acid-reactive substances (TBARS) concentrations were unaffected by any of the treatments after one day of aging (*P*>0.05), although after eight days the mineral source / vitamin E interaction did have an effect (*P*<0.05; Table 5). Values for TBARS increased four- to six-fold in the chelated minerals treatments, and from eight-to over 14-fold in the inorganic minerals treatments. The difference in TBARS activity between one and eight days' aging is due to fat peroxidation, which increased as the meat samples aged under refrigeration. At day eight, the difference between the inorganic only treatment (T1) and the chelated only treatment (T3) may have been caused by the difference in the concentration of cofactors (e.g. Se) stored in the tissue. For example, in one study muscle Se concentrations were higher in cattle fed chelated minerals' supplements<sup>(46)</sup>. Vitamin E also prevents oxidative deterioration by neutralizing the effect of free radicals<sup>(8,37)</sup>; in the present results this effect can be seen in the lower TBARS activity in the treatments containing vitamin E.

#### **Catalase**

Activity of the CAT enzyme was higher in the chelated minerals treatment at both aging times (P<0.05; Table 5), but vitamin E and the mineral source / vitamin E interaction had no effect on this variable. Apparently the increased CAT activity was associated with the higher bioavailability of chelated minerals such as Cu. This element participates, via ceruloplasmin, in oxidation of Fe from the heme group, a CAT cofactor, in this enzyme's first action stage on hydrogen peroxide<sup>(17)</sup>. The higher CAT activity in this treatment also helped to reduce fat oxidation.

## Glutathione peroxidase

Neither mineral source, vitamin E nor their interaction affected GPX activity. In contrast, this activity was lower at one days' aging than at eight days' (P<0.05; Table 5). This is related to free radical activity which increases with time due to meat exposure to the environment and bacterial multiplication. A previous study also found no effect on GPX activity in response to mineral source (chelated or inorganic) and presence or absence of vitamin E in beef cattle, and concluded that feed Se concentrations were sufficient to meet the requirements in all treatments<sup>(8)</sup>.

# **Conclusions and implications**

Supplementation with inorganic and chelated minerals (Cu, Se, and Zn), with or without vitamin E, modified quality characteristics and oxidative stability in beef lower shear force in the tested cutlets. This is probably associated with the higher absorption and bioavailability of chelated minerals compared to inorganics, which may affect Cu, Se, and Zn concentrations in the meat, as well as pH. Water holding capacity, shear force and CAT enzyme activity were consequently affected. An interaction was observed between vitamin E and mineral source on TBARS in which use of inorganic minerals

without vitamin E allowed greater oxidation in the meat. The combination of chelated minerals and vitamin E produced lower shear force values, higher water holding capacity and greater oxidative stability. All are desirable in the meat industry since they add value to meat products. Producers that grow and finish cattle would therefore benefit from supplementing finishing rations with chelated Se, Cu and Zn in conjunction with vitamin E.

# Acknowledgements

Technical and financial support was recieved from the Programa de Maestría y Doctorado en Ciencias de la Producción y de la Salud Animal de la UNAM, Consejo Nacional de Ciencia y Tecnología (CONACyT), Rancho Santa Rita, Alltech de México, DSM México y Nutrimentos Minerales de Hidalgo.

#### Literature cited:

- 1. McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA. Nutrición animal. 6a ed. Zaragoza, España: Acribia; 2002.
- 2. Greene LW, Lunt DK, Byers FM, Chirase NK, Richmond CE, Knutson RE, Schelling GT. Performance and carcass quality of steers supplemented with zinc oxide or zinc methionine. J Anim Sci 1988;(66):1818-1823.
- 3. Holder VB, Jennings JS, Swingle RS. Effects of the EPNIX<sup>TM</sup> beef program on feedlot performance in diets containing no monensin or tylosin. J Anim Sci 2016;94(Suppl 5):199.
- 4. Spears JW, Kegley EB. Effect of zinc source (zinc oxide *vs* zinc proteinate) and level on performance, carcass characteristics, and immune response of growing and finishing steers. J Anim Sci 2002;(80):2747–2752.
- 5. Edens FW. Potential for organic selenium to replace selenite in poultry diets. Zootec Int 1997;20(1):28-31.
- 6. Zhan X, Wang M, Zhao R, Li W, Xu Z. Effects of different selenium source on selenium distribution, loin quality and antioxidant status in finishing pigs. Anim Feed Sci Technol 2007;(132):202-211.

- 7. Birnie J, Farmer L, Moss B, Tollan E, Devlin D, Tollerton J, Graham B, Fearon A, Hagan T, Majury L, Gordon A. Selenium source and vitamin E: impact on beef quality. Science and technology in the feed industry. 26th International Symposium Alltech. 2010:16-19.
- 8. O'Grady MN, Monahan FJ, Fallon RJ, Allen P. Effects of dietary supplementation with vitamin E and organic selenium on the oxidative stability of beef. J Anim Sci 2001;(79):2827–2834.
- 9. Fairweather-Tait SJ, Collings R, Hurst R. Selenium bioavailability: current knowledge and future research requirements. Am J Clin Nutr 2010;91(Suppl 5):1484S–1491S.
- 10. Gressley TF. Zinc, copper, manganese, and selenium in dairy cattle rations. Proc 7th Ann Mid-Atlantic Nutrition Conf. 2009:65-71.
- 11. Cozzi G, Prevedello P, Stefani AL, Piron A, Contiero B, Lante A, Gottardo F, Chevaux E. Effect of dietary supplementation with different sources of selenium on growth response, selenium blood levels and meat quality of intensively finished Charolais young bulls. Animal 2011;5(10):1531–1538.
- 12. McDonald M, Mila I, Scalbert A. Precipitations of metal ions by plant polyphenols: optimal conditions and origin of precipitation. J Agric Food Chem 1996;(44):599-606.
- 13. Spears JW. Trace mineral bioavailability in ruminants. J Nutr 2003;133(Suppl 1):1506S-1509S.
- 14. Waldron KJ, Rutherford JC, Ford D, Robinson NJ. Metalloproteins and metal sensing. Nature 2009;(460):823-830.
- 15. Sunde RA. Selenium. In: O'Dell BL, Sunde RA. Handbook of nutritionally essential mineral elements. NY USA: Marcel Dekker; 1997:493–556.
- 16. Behne D, Kyriakopoulos A. Mammalian selenium-containing proteins. Annu Rev Nutr 2001;(21):453–473.
- 17. Suttle N. Mineral nutrition of livestock. 4th ed. CABI. Oxfordshire, UK. 2010.
- 18. Sales J, Koukolová V. Dietary vitamin E and lipid and color stability of beef and pork: Modeling of relationships. J Anim Sci 2011;(89):2836–2848.
- 19. NOM-051-ZOO-1995. "Trato humanitario en la movilización de animales", publicada en el Diario Oficial de la Federación el 26-marzo-1996.
- 20. NOM-033-ZOO-1995. "Sacrificio humanitario de los animales domésticos y silvestres", publicada en el Diario Oficial de la Federación el 07-Julio-1995.

- 21. CIE. Commission Internationale de L'Eclairage. Technical report, colorimetry. 2004.
- 22. NMX-F-317-S-1978. "Determinación de pH en los alimentos". 23-Mayo-1978.
- 23. Hunt MC, Acton JC, Benedict RC, Calkins CR, Cornforth DP, Jeremiah LE, Olson DG, *et al.* Guidelines for meat color evaluation. AMSA. 1991.
- 24. Mancini RA, Hunt MC. Current research in meat color. Meat Sci 2005;(71):100-121.
- 25. Guerrero I, Ponce E, Pérez ML. Curso práctico de tecnología de carnes y pescado. Universidad Autónoma Metropolitana. DF, México. 2002.
- 26. Tomás MC, Funes J. Application of 2-Thiobarbituric acid reaction to exudates of frozen and refrigerated meats. J Anim Sci 1987;(52):575-579.
- 27. Hernandez P, Zomeño L, Ariño B, Blasco A. Antioxidant, lipolytic and proteolytic enzyme activities in pork meat from different genotypes. Meat Sci 2004;(66):525-529.
- 28. Honikel KO. Reference methods for the assessment of physical characteristics of meat. Meat Sci 1998;(49):447-457.
- 29. AMSA. American Meat Science Association. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. 2nd ed, version 1.0. 2015.
- 30. Kastner CL, Schwenke JR, Kenney PB, Campbell RE, Kendall JA, Milliken GA. Comparisons of the effect of electrical stimulation methods on *postmortem* pH decline in beef muscle. Meat Sci 1993;(35):183-190.
- 31. Smulders FJM, Toldrá F, Flores J, Prieto M. New technologies for meat and meat products. Utrecht: Audet Tijdschriften 1992;(182):186–188.
- 32. Page JK, Wulf DM, Schwotzer TR. A survey of beef muscle color and pH. J Anim Sci 2001;(79):678–687.
- 33. Gatellier P, Hamelin C, Durand Y, Renerre M. Effect of a dietary vitamin E supplementation on colour stability and lipid oxidation of air- and modified atmosphere-packaged beef. Meat Sci 2001;(59):133–140.
- 34. Kanner J. Oxidative processes in meat and meat products: quality implications. Meat Sci 1994;(36):169–189.
- 35. Lanari MC, Schaefer DM, Liu Q, Cassens RG. Kinetics of pigment oxidation in beef from steers supplemented with vitamin E. J Food Sci 1996;(61):884–889.

- 36. McDowell LR. Vitamins in animal and human nutrition, 2nd ed. USA: Iowa State University Press; 2000.
- 37. Mitsumoto M, Arnold RN, Schaefer DM, Cassens RG. Dietary vitamin E supplementation shifted weight loss from drip to cooking loss in fresh beef longissimus during display. J Anim Sci 1995;(73):2289-2294.
- 38. Brewer MS. Water-Holding Capacity. In: Encyclopedia of meat sciences, Vol 1. Elsevier Ltd. USA. 2014:274-282.
- 39. Shackelford SD, Morgan JB, Cross HR, Savell JW. Identification of threshold levels for Warner-Bratzler shear force in beef top loin steaks. J Muscle Foods 1991;(2):289-296.
- 40. Monin G, Santé-Lhoutellier V. Color and texture deviations. In: Encyclopedia of meat sciences, Vol 1. Elsevier Ltd. USA. 2014:339-345.
- 41. Maddock KR, Huff-Lonergan E, Rowe LJ, Lonergan SM. Effect of pH and ionic strength on μ- and m-calpain inhibition by calpastatin. J Anim Sci 2005;(83):1370–1376.
- 42. Grayson AL. Effect of degree of dark-cutting on tenderness and flavor attributes of beef [Tesis doctoral]. EU, Texas: Texas A&M University; 2014.
- 43. Dutson TR. Relationship of pH and temperature to distruption of specific muscle proteins and activity of lysosomal proteases. J Food Biochem 1983;(7):223-245.
- 44. Huff-Lonergan E, Lonergan SM. Mechanisms of water-holding capacity of meat: The role of *postmortem* biochemical and structural changes. Meat Sci 2005;(71):194-204.
- 45. Pietrasik Z, Janz JA. Influence of freezing and thawing on the hydration characteristics, quality, and consumer acceptance of whole muscle beef injected with solutions of salt and phosphate. Meat Sci 2009;(8):523–532.
- 46. Cravo AS, Veiga M, Aferri G, Pereira da Silva RR, da Luz S, de Freitas JE, Leme PR, Palma F. Lipid and selenium sources on fatty acid composition of intramuscular fat and muscle selenium concentration of Nellore steers. R Bras Zoot 2012; 41(11):2357-2363.