

## Supplementary Feeding of Economas E<sup>®</sup> Improves Storage Life of Hanwoo Beef

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### ABSTRACT

Vitamin E has been applied as a dietary supplement or post mortem to meat cuts to preserve meat quality and extend shelf life. This study was conducted to determine the effects of supplementation of the diet of Hanwoo steers with Economas E<sup>®</sup>, a less expensive alternative to vitamin E, on meat quality preservation. To accomplish this, 36 Hanwoo steers were randomly allotted into three treatment groups; no additive (control), Economas E<sup>®</sup> (T1) and vitamin E (T2). Vitamin E and Economas E<sup>®</sup> were included in the diets at 500 mg/head and 200 mg/head, respectively, for 5 months. Evaluation of carcass performance parameters immediately after slaughter revealed no treatment effects ( $P>0.05$ ). Samples collected from the loin area and stored at 4°C for up to 9 days showed that T1 and T2 preserved pigment and lipid stability as indicated by significantly ( $P<0.05$ ) higher CIE L\* and lower CIE a\* and CIE b\* values and a less rapid increase in thiobarbituric acid reactive substance (TBARS) relative to the control. However, treatments had no effect ( $P>0.05$ ) on cholesterol and fatty acid content in meat. Based on these findings, Economas E<sup>®</sup> provided at 200 mg/head is as effective as vitamin E applied at 500 mg/head at preserving Hanwoo meat quality over a 9 day storage period at 4°C.

**(Key words :** Antioxidant, Hanwoo, Meat color, Meat preservation, Vitamin E)

### INTRODUCTION

The meat quality preservative properties of vitamin E have been thoroughly investigated and it has been well established that  $\alpha$ -tocopherol is the most potent form of natural vitamin E for these purposes (Sales and Koukolova, 2011). Mitsumoto et al. (1993) revealed that dietary supplementation with vitamin E is more effective at extending the shelf-life of meat than postmortem treatment of the meat. However, its prohibitive cost has led to the formulation of relatively cheaper alternative feed additives.

Lipid and pigment oxidation in stored meat affects quality traits including color display life, flavor and texture. Deterioration of these qualities can result in substantial economic losses (Sales and Koukolova, 2011). Meat color is the most important factor in determining consumer preference when buying meat products, especially that of red meats such as beef. A cherry bright red color indicates fresh meat however, pigment oxidation during storage results in a brown color replacing the cherry bright red color. This brown pigment, which is known as metmyoglobin, is evident in meat after storage for several hours to several days. MetMb together with two other pigments, oxymyoglobin (OxyMb) and

deoxymyoglobin (DeoxyMb), affect beef color (Liu et al., 1995). DeoxyMb is the purple pigment observed in freshly cut meat. After a few minutes of exposure to air, DeoxyMb is oxygenated to OxyMb, giving beef its characteristic bright cherry-red color. However, further oxidation of OxyMb results in formation of the brown pigment, MetMb (Liu et al., 1995). Consumers discriminate against meat cuts that lack a fresh appearance (Kropf et al., 1986). Studies have confirmed that vitamin E (tocopherol) extends the positive color display of beef. Lipid oxidation, predominantly of unsaturated fatty acids, results in rancidity in uncooked meat and (or) a warmed-over flavor in cooked meat, which is the major cause of deterioration in flavor and texture. Lipid oxidation is also positively correlated with pigment oxidation, although the basis for the observed relationship is not yet clearly understood. Liu et al. (1995) postulated that radicals generated from lipid oxidation directly promote pigment oxidation and (or) indirectly damage pigment-reducing systems. Vitamin E is a lipid soluble vitamin that is widely applied for its antioxidant properties in biological systems (McCay and King, 1980). Animals cannot synthesize this vitamin hence, it has to be supplied through dietary provisions (Faustman et al., 1989). Several previous studies have investigated the effects

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of supplemental vitamin E provided to beef cattle on meat quality and it has been shown to delay browning in fresh cut meats from various cattle breeds (Arnold et al., 1993). Supplemental vitamin E was also found to stabilize lipid oxidation during storage (Faustman et al., 1989).

The objective of this study was to investigate the preservative effect of Economas E<sup>®</sup>, a less expensive commercial feed additive, for its potential to replace vitamin E, in Hanwoo (Korean native beef breed) meat.

## MATERIALS AND METHODS

### 1. Additives

A commercially available vitamin E replacement, Economas E<sup>®</sup>, was purchased from Alltech Co. USA. This product is a blend of vitamin C, selenium and algae that has a cost one-third of that of commercial natural vitamin E supplements. Natural vitamin E in the form of  $\alpha$ -tocopheryl acetate was purchased from Roche Vitamin Co. (France).

### 2. Animal and experimental design

A total of 36 Hanwoo steers were randomly assigned to three treatment groups; no additive (control), T1 (Economas E<sup>®</sup>) and T2 (vitamin E). Each experimental group consisted of three stalls and four herds per stall. All steers were fed with fattening concentrate diet, with or without an additive, and rice straw *ad libitum*. Steers in the control group were fed the commercial concentrate without any additive, while T1 and T2 steers were fed the concentrate with a top dressing supplementation of Economas E<sup>®</sup> (200 mg/head) and vitamin E (500 mg/head), respectively. The animals were fed twice a day and had free access to water. The feeding trial lasted 5 months.

### 3. Sampling and analysis

At the end of the 5 months, the steers were slaughtered and carcass performance parameters (thickness of back fat, area of back fat and carcass weight) were investigated. A sample was collected from the loin area of each carcass immediately after slaughter, and the split carcasses were stored in a chilling room (air temperature =  $0 \pm 1^\circ\text{C}$ ) for 24 h and then vacuum packed. Prepared meat samples were subsequently stored at  $4^\circ\text{C}$  in a refrigerator for 9 days, after which the

following meat quality parameters were determined using the loin meat; pH, color, thiobarbituric acid reactive substance (TBARS) content, volatile basic nitrogen (VBN) content, cholesterol content and fatty acid profiles.

### 4. pH values

Ten grams of sample meat and 90 mL of distilled water were mixed and homogenized using a homogenizer (NS-50, Japan). The pH of the homogenate was then measured using a pH meter (520A, Orion Research Inc., USA).

### 5. Meat color

The color of the sample meat was determined using a Chromameter (CR301, Minolta Co., Germany). The characteristics of color were divided into three categories, brightness (CIE L\*), redness (CIE a\*) and yellowness (CIE b\*). A white tile standard color board consisting of  $Y = 92.40$ ,  $x = 0.3136$ ,  $y = 0.3196$  was used as a reference.

### 6. Thiobarbituric acid reactive substance (TBARS) values

Thiobarbituric acid reactive substance content in sample meat was determined according to Witte et al. (1970). Briefly, 20 g of meat and 50 mL of 20% TCA (trichloroacetic acid, in 2 M phosphate) were mixed and homogenized using a tissue grinder (1102-1, Japan). The slurry was then filtered through filter paper (Whatman No. 1), after which the filtrate was diluted to 100 mL with distilled water. Five milliliters of prepared filtrate were then mixed with 5 mL of 5 mM thiobarbituric acid reagent and allowed to react for 15 h at room temperature. At the end of the reaction period, the optical density was measured at 530 nm using a spectrophotometer (Sequoia-Turner Co., USA). TBARS were calculated by powering 5.2 to observed optical density values.

### 7. Volatile basic nitrogen (VBN) values

Volatile basic nitrogen content in sample meat was determined according to Lee et al. (2011). Briefly, 10 g of meat sample was mixed with 90 mL distilled water, homogenized and then filtered through filter paper (Whatman No 1). One milliliter of filtrate was placed into the outside room of a Conway unit, while the inside room was filled with 1 mL of 0.01 N boric acid and three drops of indicator

mixture (0.066% methyl red + 0.066% bromocresol green). The joint area with a cover was sealed with glycerin and 1 mL of 50% K<sub>2</sub>CO<sub>3</sub> was added to the outside room of the Conway unit immediately after closing the cover. The unit was then gently mixed horizontally and incubated at 37°C for 120 min. After incubation, the alkalinity of boric acid was measured by titration using 0.02N H<sub>2</sub>SO<sub>4</sub>. VBN values were calculated as follows:

$$\text{VBN} = ((a-b)*F*28.014*100)/\text{sample weight}$$

where a is the amount of H<sub>2</sub>SO<sub>4</sub> consumed, b is the H<sub>2</sub>SO<sub>4</sub> used for the blank sample, F is the factor for 0.02 N H<sub>2</sub>SO<sub>4</sub>, and 28.014 is a constant to represent the amount of nitrogen required to consume 1 mL of 0.02 N H<sub>2</sub>SO<sub>4</sub>.

## 8. Cholesterol content

The cholesterol content in meat was determined according to Rule et al. (2002). Briefly, 1 g of freeze dried meat sample was mixed with 3 mL of ethanol and 1 mL of 33% KOH. The mixture was then heated at 85°C in a water bath for 60 h. After cooling to room temperature, 3 mL of distilled water, 2 mL of hexane and 1 mL of stigmasterol were added. The upper phase of the mixture was separated and used as a sample for gas chromatography (GC) analysis (Clarus 500, Perkin Elmer Life and Analytical Science, Shelton, USA) to measure the cholesterol.

## 9. Long chain fatty acids profiles

Lipid extraction and methylation of fatty acid were performed as described by Folch et al. (1957) and Park and Goins (1994), respectively. Briefly, 0.5 g of extracted lipid sample was mixed with 2 mL of solvent mixture consisting of methanol and benzene (4:1, v/v) and 0.2 mL of acetyl chloride. The sample was then heated at 100°C using a heating block for 1 h, after which the mixture was cooled to room temperature and 1 mL of hexane and 5 mL of 6% potassium carbonate were added. The mixture was then centrifuged at 3,000 rpm for 15 min, after which 0.5 µL of

the supernatant was analyzed for fatty acids by gas chromatography (GA-17A, Shimadzu, Japan).

## 10. Statistical analysis

The effects of treatments on carcass performance parameters (meat pH, meat color, TBARS, VBN, cholesterol and fatty acids profiles) were determined by GLM (general linear model). Duncan's multiple range tests were performed to separate the means. All statistical analyses were conducted using the SPSS statistical program (version 18, IBM, USA) and the statistical confidence interval was set at 95%.

# RESULTS AND DISCUSSION

## 1. Carcass performance

There were no significant differences in back fat thickness, area of back fat and carcass weight among treatments ( $P > 0.05$ ), although T1 and T2 tended to have lower carcass weights and back fat thickness (Table 1). These findings are in agreement with those of earlier investigations in which no effects on carcass performance were associated with vitamin E supplementation (Liu et al., 1995).

## 2. Meat color

The effects of treatments on color stability of stored sample meat are shown in Table 2. Meat color is the primary characteristic used by consumers to judge freshness of meat cuts accordingly, it is important to delay meat discoloration in meat. The lightness (CIE L\*) of T2 was consistently significantly higher than that of the other samples throughout the storage period. There were no differences observed between the control and T1 group from day 1 until day 3. However, at days 5 and 9 T1 meat samples had higher CIE L\* figures ( $P < 0.05$ ) than the control group. There were no significant effects of either storage day or treatment by storage interaction ( $P > 0.05$ ). The initial redness (CIE a\* value)

Table 1. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on carcass performance parameters of Hanwoo steers

Treatment	Thickness of back fat (mm)	Area of back fat (cm <sup>2</sup> )	Carcass weight (kg)
C	13.56 ± 3.72	85.11 ± 7.19	421.44 ± 34.47
T1	11.11 ± 3.14	84.78 ± 6.66	400.78 ± 37.40
T2	11.22 ± 3.55	87.78 ± 7.10	400.33 ± 40.92

Table 2. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on color of loin meat of Hanwoo steers during storage

Color	Treatment				Significance <sup>1)</sup>	
	Day	C	T1	T2	SEM	P
L	0	41.26 <sup>a</sup>	42.17 <sup>a</sup>	44.72 <sup>b</sup>	0.426	***
	1	42.51 <sup>a</sup>	42.04 <sup>a</sup>	46.29 <sup>b</sup>	0.454	***
	3	42.08 <sup>a</sup>	42.92 <sup>a</sup>	46.91 <sup>b</sup>	0.401	***
	5	41.21 <sup>a</sup>	43.11 <sup>b</sup>	45.48 <sup>c</sup>	0.362	***
	9	41.76 <sup>a</sup>	43.65 <sup>b</sup>	46.05 <sup>c</sup>	0.361	***
Significance	Treatment			***		
	Day			NS		
	Interaction			NS		
Color	Day	C	T1	T2	SEM	P
a	0	19.90 <sup>b</sup>	13.94 <sup>a</sup>	13.17 <sup>a</sup>	0.344	***
	1	21.25 <sup>c</sup>	17.76 <sup>b</sup>	12.95 <sup>a</sup>	0.424	***
	3	19.82 <sup>c</sup>	16.85 <sup>b</sup>	13.19 <sup>a</sup>	0.419	***
	5	18.21 <sup>b</sup>	16.56 <sup>b</sup>	13.21 <sup>a</sup>	0.404	***
	9	18.13 <sup>b</sup>	15.61 <sup>a</sup>	14.74 <sup>a</sup>	0.337	***
Significance	Treatment			***		
	Day			*		
	Interaction			***		
Color	Day	C	T1	T2	SEM	P
b	0	13.70 <sup>b</sup>	10.67 <sup>a</sup>	11.51 <sup>a</sup>	0.268	***
	1	15.35 <sup>b</sup>	13.40 <sup>a</sup>	12.99 <sup>a</sup>	0.324	*
	3	14.57 <sup>b</sup>	13.38 <sup>a</sup>	13.26 <sup>a</sup>	0.177	**
	5	14.07	13.91	13.7	0.199	NS
	9	14.57	13.63	14.25	0.179	NS
Significance	Treatment			***		
	Day			***		
	Interaction			*		

<sup>1)</sup> NS, \*, \*\*, and \*\*\* indicate not significant, P<0.05, P<0.01, and P<0.001, respectively.

<sup>a,b,c</sup> Values with different superscripts in the same column are significantly different (P<0.05).

was significantly higher in the control group than the other treatment groups, which may have been related to the slight differences in fat content observed between the treatment groups. Overall, treatment, storage period and their interaction had significant effects (P<0.05) on the CIE a\* value. These findings suggest that the experimental treatments do not share the same patterns for redness color changes during the storage period. These color preservation effects of supplementing diets with vitamin E or its alternative have been confirmed in other studies (Lee et al., 2003; Lee et al., 2008). In a study conducted by Arnold et al. (1993), meat samples from steers that received supplemental vitamin E showed delayed metmyoglobin (MetMb) formation. During the storage periods, the redness in the control group decreased significantly

relative to the treatment groups (P<0.05). Discoloration in the control was apparent with increased storage. Yellowness in T1 and T2 meat samples was significantly higher (P<0.05) than that of the control group from the day of slaughter to day 3 of storage. From day 5 of storage to the end of the storage period, there were no significant differences between treatments (P<0.05). The same trend was noted in another study conducted by Lee et al. (2008). There was an overall effect of treatment, storage period and their interaction (P<0.05).

Pigment oxidation has been reported to be initiated in phospholipid-rich membranes (Mitsumoto et al., 1993), and the incorporation of vitamin E, a soluble antioxidant that can permeate and be incorporated into these membranes, has

been shown to be effective at stabilizing meat color in storage.

### 3. Meat pH values

Treatments had a significant effect on pH ( $P < 0.001$ ). Vitamin E resulted in the highest pH values over the storage period, followed by Economas E<sup>®</sup> and then the non-supplemented diet from day 1 to day 9 (Table 3). At slaughter (day 0), there was no significant difference between treatments ( $P > 0.05$ ). The observed effects of either vitamin E or Economas E<sup>®</sup> dietary supplementation on meat pH are contrary to results from other related studies in which vitamin E supplementation had no effect on meat pH during storage (Juarez et al., 2012; Liu et al., 1995).

### 4. Thiobarbituric acid reactive substance (TBARS) values

There were significant treatment  $\times$  day interaction effects on TBARS values ( $P < 0.01$ ). While TBARS values for the control

and T2 increased from the initial values at slaughter, those for T1 declined at day two before they began increasing from day 4 to day 9 (Table 4). The Economas E<sup>®</sup> group tended to have lower values than the other treatment groups. There were no differences between day 5 and 9. This observation is in agreement with the lipid stabilizing effect of vitamin E in other studies of Hanwoo meat (Lee et al., 2003; Lee et al., 2008) and in other breeds (Arnold et al., 1993; Faustman et al., 1989).

### 5. Volatile basic nitrogen (VBN) values

In this study, all groups showed increased VBN with increasing storage days (Table 5). However, the VBN values of all groups were within the range of values considered to represent fresh meat. According to Korean food safety standards, when the VBN is below 20 mg%, the meat is regarded as fresh (Min et al., 2007).

### 6. Cholesterol and fatty acids profiles

Table 3. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on the pH of loin area meat of Hanwoo steers during storage

Treatment	Storage periods, days					Significance <sup>1)</sup>		
	0	1	3	5	9	T	D	I
C	5.47	5.43 <sup>a</sup>	5.44 <sup>a</sup>	5.48 <sup>a</sup>	5.51 <sup>a</sup>			
T1	5.54	5.56 <sup>b</sup>	5.67 <sup>b</sup>	5.67 <sup>b</sup>	5.68 <sup>b</sup>			
T2	5.70	5.71 <sup>c</sup>	5.82 <sup>c</sup>	5.82 <sup>b</sup>	5.91 <sup>c</sup>	***	**	NS
SEM	0.04	0.02	0.02	0.03	0.03			
P value	0.11	0.00	0.00	0.00	0.00			

<sup>1)</sup> T, D, and I are effects of treatment, days and treatment  $\times$  days interaction, respectively; NS, \*, \*\*, and \*\*\* indicate not significant,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively.

<sup>a,b,c</sup> Values with different superscripts in the same column are significantly different ( $P < 0.05$ ).

Table 4. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on thiobarbituric acid reactive substance (TBARS) values of loin meat of Hanwoo steers during storage

Treatment	Storage periods, days					Significance <sup>1)</sup>		
	0	1	3	5	9	T	D	T $\times$ D
C	0.15 <sup>a</sup>	0.20 <sup>a</sup>	0.33 <sup>a</sup>	0.44	0.62			
T1	0.29 <sup>b</sup>	0.24 <sup>b</sup>	0.25 <sup>ab</sup>	0.42	0.58			
T2	0.31 <sup>b</sup>	0.36 <sup>c</sup>	0.42 <sup>b</sup>	0.41	0.60	***	***	***
SEM	0.012	0.007	0.017	0.018	0.02			
P value	<0.001	<0.001	0.004	0.798	0.796			

<sup>1)</sup> T, D, and I are effects of treatment, days and treatment  $\times$  days interaction, respectively; NS, \*, \*\*, and \*\*\* indicate not significant,  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively.

<sup>a,b,c</sup> Values with different superscripts in the same column are significantly different ( $P < 0.05$ ).

Table 5. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on the volatile basic nitrogen of loin meat of Hanwoo steers during storage

Treatment	Storage periods, days					Significance <sup>1)</sup>		
	0	1	3	5	9	T	D	I
C	12.6	14.98 <sup>b</sup>	15.4	16.94 <sup>ab</sup>	19.04			
T1	12.6	15.26 <sup>b</sup>	15.82	18.48 <sup>b</sup>	18.62			
T2	9.8	13.58 <sup>a</sup>	14.7	15.96 <sup>a</sup>	17.08	***	***	NS
SEM	0.808	0.217	0.244	0.304	0.38			
P value	0.311	0.025	0.221	0.024	0.141			

<sup>1)</sup> T, D, and I are effects of treatment, days and treatment × days interaction, respectively; NS, \*, \*\*, and \*\*\* indicate not significant, P<0.05, P<0.01, and P<0.001, respectively.

<sup>a,b,c</sup> Values with different superscripts in the same column are significantly different (P<0.05).

Table 6. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on cholesterol contents of loin area meat of Hanwoo steers

Treatment	Cholesterol, mg/100 g
C	50.58 ± 4.22
T1	47.40 ± 4.43
T2	51.80 ± 3.52

Table 7. Effects of dietary supplementation with Economas E<sup>®</sup> (T1), vitamin E (T2) and a non-supplemented diet (C) on fatty acid composition in loin area meat of Hanwoo steers

Fatty acids	Treatment		
	C	T1	T2
Lauric acid	0.10±0.00	0.10±0.00	0.10±0.00
Myristic acid	4.18±0.33	4.45±0.79	4.38±0.89
Palmitic acid	33.95±2.55	35.23±2.33	34.90±5.31
Stearic acid	17.98±1.66	16.38±2.48	17.05±2.43
Arachidic acid	0.10±0.00	0.10±0.00	0.13±0.04
Sum of saturated fatty acids	56.30±3.95	56.25±4.53	56.55±7.88
Myristoleic acid	0.65±0.15	0.78±0.20	0.68±0.29
Palmitoleic acid	2.83±0.37	3.33±0.75	2.83±0.54
Oleic acid	36.88±3.39	36.23±3.73	36.55±7.06
Linoleic acid	1.13±0.22	1.45±0.27	1.43±0.39
Linolenic acid	0.10±0.00	0.10±0.00	0.10±0.00
Gadoleic acid	0.23±0.08	0.20±0.00	0.15±0.05
Eicosatrienoic acid	0.48±0.18	0.38±0.13	0.38±0.15
Sum of unsaturated fatty acid	42.28±3.92	42.45±4.70	42.10±8.06
Unknown	1.43±0.19	1.30±0.19	1.35±0.22
Total	100	100	100

Mean ± standard deviation.

There was no significant difference (P>0.05) in cholesterol (Table 6) and long chain fatty acids (Table 7) among treatment groups. In a study conducted by Liu et al. (1995), vitamin E supplementation did not affect carcass characteristics or quality, which is in accordance with the results of the present study.

## CONCLUSIONS

Economas E<sup>®</sup>, a commercial alternative to vitamin E, showed relatively similar effects on color and preservation qualities of Hanwoo steer meat as vitamin E when provided at a lower level. Since the price of Economas E<sup>®</sup> is one third that of vitamin E, it could be an appropriate substitute to vitamin E as a preservative for meat color and other preservative qualities in beef.

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