

## Effect of vitamin E supplementation and pre-rigor injection with crude kiwifruit extract on physico-chemical and sensory traits of Karadi sheep

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### Abstract

The study was designed to examine the effects of both dietary vitamin E supplementation and pre-rigor injection with crude kiwifruit extract on some physico-chemical and sensory traits of Karadi sheep. Rams were divided equally into two treatments (T) groups. T<sub>1</sub> group was fed a control diet and T<sub>2</sub> group was supplemented with vitamin E. After exsanguination of rams and dressing carcasses, the three major muscles namely *longissimusdorsi* (LD), *semimembranosus* (SM) and *biceps femoris* (BF) were removed from both T1 and T2 carcasses, Then injected with crude kiwifruit extract at concentrations of 8 and 12% and distilled water at a rate of 10 % muscle weight. The other treatment is considered as control. The muscles samples were packed in polyethylene bags and stored at 4°C for 24hr, then kept frozen at -18°C, until the time analysis. The results showed that vitamin E alone or its combinations with injection with crude kiwifruit extracts (8 and 12%) were more effective in retarding lipid oxidation and of met-myoglobin formation with lower (p<0.05) cooking loss percentages and higher (p<0.05) moisture content and their reflection on improvement water holding capacity and sensory traits in LD, SM and BF muscles as compared with control and other treatments. LD muscle was more effective in maintenance the traits above than SM and BF muscles. It can be concluded that the presence of vitamin E in muscle together with crude Kiwifruit extract played a key role in retarding lipid oxidation and meat color, also improvement of sensory traits.

Key words: Vit. E, Kiwi fruits extract, Physico- chemical and sensory traits, sheep meat

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تأثير إضافة فيتامين E والحقن بعد الذبح مباشرة مع المستخلص الخام لفاكهة الكيوي في بعض

الصفات الفيزيوكيميائية والحسية للحوم الأغنام الكرادية

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### الخلاصة

صممت هذه الدراسة لفحص تأثيرات كل من إضافة فيتامين E في العليقة والحقن بعد الذبح مباشرة مع المستخلص الخام لفاكهة الكيوي في بعض الصفات الفيزيوكيميائية والحسية للحوم الأغنام الكرادية. قسمت الكباش إلى معاملتين (T) (مجموعتين). غذيت المجموعة T1 على عليقة سيطرة والمجموعة T2 غذيت على عليقة سيطرة+ فيتامين E. بعد الذبح واستنزاف الدم للكباش تم تجهيز الذبائح، وتم فصل ثلاثة عضلات رئيسية وهي العضلة الظهرية الطويلة (*Longissimus dorsi* (LD) والعضلة نصف الغشائية (*Semimembranosus* (SM) وعضلة الفخذ ثنائية الراس (*Biceps femoris* (BF) لكلا المجموعتين T1 و T2 للذبائح، تم حقن هذه العضلات مع المستخلص الخام لفاكهة الكيوي بتركيز 8 و 12% والماء المقطر وبمعدل حقن 10% من وزن كل عضلة. وعدت المعاملة الأخرى معاملة سيطرة. حفظت عينات العضلات المعاملة في أكياس من البولي اثيلين. وخرزت في

التبريد بدرجة حرارة 4 م° لمدة 24 ساعة، ثم حفظت بالتجميد في درجة حرارة -18 م° لحين إجراء التحاليل. أشارت النتائج بان المعاملة مع فيتامين E لوحده أو اتحاده مع المستخلص الخام لفاكهة الكيوي بتركيز 8 و 12% كانت أكثر كفاءة في أعاقه أكسدة الدهون وتكوين صبغة الميتامايوغلوبين مع أوطأ ( $p < 0.05$ ) فقدان عند الطبخ وأعلى ( $p < 0.05$ ) محتوى رطوبي وانعكس ذلك في تحسين قابلية اللحم على حمل الماء والصفات الحسية في العضلات LD، SM و BF مقارنة مع معاملة المقارنة والمعاملات الأخرى. كانت العضلة LD أكثر كفاءة في إدامة الصفات أعلاه مقارنة مع العضلات SM و BF. ويمكن الاستنتاج بان وجود فيتامين E في العضلة مع المستخلص الخام لفاكهة الكيوي قد لعبت دوراً في أعاقه أكسدة الدهون ولون اللحم وكذلك تحسين الصفات الحسية للحم الكباش الكرادية.

الكلمات المفتاحية: فيتامين E، مستخلص فاكهة الكيوي، الصفات الفيزيوكيميائية والحسية، لحم الأغنام

## Introduction

Meat eating qualities including tenderness, juiciness, meat colour and flavour have been considered as the most important palatability traits by consumers (1). Among these traits, tenderness has been rated as the most important meat quality characteristic (2). The technologies used to improve meat tenderness have achieved success to various degrees, which included post-mortem ageing (3), mechanical tenderization (4, 5), electrical stimulation (6), ionic chemical solution (7) and addition of plant enzymes such as ficin, papain and bromelain (8). The enzymatic tenderizing treatment with kiwifruit extract could be a feasible approach for the meat industry due to mild tenderizing effects of actinidin, a cysteine protease in kiwifruit extract compared with other plant proteases (9). However, other quality attributes such as colour, drip and cooking loss and lipid stability, hard to be maintained or preserved, which impose an adverse effect to consumers. Therefore, it is hoped that dietary vitamin E supplementation (as antioxidant). May act to break the chain of lipid oxidation in cell membranes, prevents the formation of products causing off-flavors, and finally preserve quality characteristics of meat (10). The present study is designed to investigate the overall impact of using both dietary vitamin E and crude kiwifruit extract and their combination on the some of physical, chemical and sensory properties of karadi sheep.

## Materials and Methods

- Experimental animals, muscle samples, crude kiwifruit extract and treatments:** Thirty two of karadi rams were divided into two groups. Group 1 were fed a commercial diet without vitamin E and used as a control diet (c), Group 2 were supplemented with 1000 mg of vitamin E per animal daily for 60 days (vit. E). After slaughtering and dressing carcasses, the carcasses were fabricated into the *longissimusdorsimuscle* (LD), *semimembranosus* muscle (SM) and *biceps femorismuscle* (BF). The treated and untreated muscles with vitamin E were injected with cold crude kiwifruit juice extract at concentration of 8 and 12% and distilled water through 30 min post-mortem at a rate of 10% muscle weight. The control (c) group (untreated with vitamin E and non-injected). After injection, the muscles were allowed to equilibrate for 30 min at room temperature. The muscle samples were packed in polyethylene bags, kept at 10°C for 5 hr before they were stored at refrigeration (4°C) for 24 hr, then stored in freezing at -18°C. The kiwifruit juice was prepared according to the described method (11). The unpeeled fruit was pressed with a bladder press. The juice was filtered through filter cloth, the filtrate was again filtered through glass wool. The filtrate juice was stored at -18°C and deforested at refrigeration before use, the filtrate juice was filtered through glass wool. The supernatant (crude enzyme extract) was diluted with cold distilled water to obtain

different concentrations at 8 and 12% crude enzyme extract. The treatments are employed: control (c), vitamin E supplementation (E), injection with distilled water (w), injection of 8 and 12 % crude kiwifruit extract(A<sub>1</sub>, A<sub>2</sub>), vit.E+A<sub>1</sub> (E+A<sub>1</sub>), vit.E+A<sub>2</sub> (E+A<sub>2</sub>) and vit.E+w (E+w).

- **Cooking loss:** Cooking loss was determined according (12). The cooking loss was calculated by the following formula:

$$\text{Cooking loss\%} = \frac{\text{Raw sample weight} - \text{cooked sample weight}}{\text{Raw sample weight}} \times 100$$

- **Moisture content:** moisture content was determined as weight loss after the samples were dried in a convection oven at 105°C for 16 hr (13).
- **Thiobarbituric acid (TBA) value:** The TBA values were determined by using solution containing 20% trichloroacetic acid (TCA) in 2M phosphoric acid according to the method described (14). The absorbance was read at 530 nm by using spectrophotometer (Shimadzu, Japan). The TBA value was expressed as mg malonaldehyde (MDA)/kg muscle, and calculated by multiplying the absorbance (A) by 5.2 factor as follows: TBA value (mg MDA/kg muscle) = A<sub>530</sub> × 5.2
- **Sensory evaluation:** The muscle samples of LD, SM and BF were evaluated for sensory attributes (colour, flavour and aroma, tenderness, juiciness and overall acceptability). Muscle samples were thawed under refrigeration for 24 hr., the muscle samples were cooked in oven at 176°C for 8.5 min until reaching the internal temperature of 70°C, then served warm at 60°C to eight trained panelists (12). Panelists rated each sample for different attributes with five point scale ranging between 1 and 5 color (1-very dark, 5-reddish brown), flavor and aroma (1-very pronounced rancid, 5-no detectable rancid), Tenderness (1-tough, 5-very good tender), juiciness (1-dry, 5-very good juicy) and overall acceptability (1-refused, 5-very good acceptable) (15).

### Results and Discussion

- **Moisture content:** The results in Table (1) showed that muscles treated with vitamin E had higher (p<0.05) moisture content (75.92, 75.48 and 75.16%) for LD, SM and BF respectively than those of control treatment (74.52, 73.65 and 72.79% respectively). Results showed that treatment E+A<sub>2</sub> resulted in a higher (p<0.05) moisture content (78.39%) in LD, (77.94%) in SM and (77.44%) in BF muscles, whereas, Control treatment recorded a lower (p<0.05) moisture content (74.52%) in LD, (73.65%) in SM and (72.79%) in BF muscles. However, E+A<sub>2</sub> and E+A<sub>1</sub> treatment had higher moisture content than those of A<sub>2</sub>, A<sub>1</sub> and other treatment for LD, SM and BF muscles, LD muscles had significantly (p<0.05) the highest moisture content as compared with SM and BF muscles (Table 1). The results showed that muscle samples treated with dietary vitamin E and their combination with kiwifruit juice extract had a higher moisture content than control and other treatments, which may be due to the synergistical action between vitamin E and kiwifruit juice enzyme (Actinidin) on muscle proteins and their action on the muscle fibre degradation for supplying charged sites available for water binding with protein (16). This result confirmed those reported by Hassan (17) who showed that injection aged sheep meat with *Calotropisprocera* protease enzyme resulted in a higher moisture content than control treatment.

**Table (1) Effect of vitamin E, kiwifruit juice extract, distilled water pre-rigor injection treatments and their combinations on moisture content in *longissimusdorsi* muscle (LD), *semimembranosus* muscle (SM) and *biceps femoris* muscle (BF) of Karadi sheep (Means  $\pm$  S.E)**

Treat. Type of M.	C	E	W	E+W	A <sub>1</sub>	A <sub>2</sub>	E+A <sub>1</sub>	E+A <sub>2</sub>
LD	74.52 $\pm$ 0.02 A A	75.92 $\pm$ 0.02 b A	74.93 $\pm$ 0.01 c A	75.59 $\pm$ 0.01 d A	76.91 $\pm$ 0.01 e A	77.44 $\pm$ 0.02 f A	77.94 $\pm$ 0.02 g A	78.39 $\pm$ 0.01 h A
SM	73.65 $\pm$ 0.02 a B	75.48 $\pm$ 0.02 b B	74.23 $\pm$ 0.02 c B	75.13 $\pm$ 0.05 d B	76.48 $\pm$ 0.02 e B	77.14 $\pm$ 0.02 f B	77.38 $\pm$ 0.02 g B	77.94 $\pm$ 0.02 h B
BF	72.79 $\pm$ 0.01 a C	75.16 $\pm$ 0.02 b C	73.57 $\pm$ 0.03 c C	74.59 $\pm$ 0.01 d C	76.17 $\pm$ 0.01 e C	76.67 $\pm$ 0.02 f C	77.18 $\pm$ 0.03 g C	77.44 $\pm$ 0.02 h C

C: control, E: vit E, W: water, E+w,

A<sub>1</sub> and A<sub>2</sub>: crude kiwifruit extract at concentrations 8 and 12 respectively.

E+A<sub>1</sub> and E+A<sub>2</sub>: vit E+A<sub>1</sub> (8%) and vit E+A<sub>2</sub> (12%).

Means having different small letters (abc..) among treatments for each muscles are significantly different (p<0.05).

Means having different capital letters (ABC) among muscles for each treatment are significantly different (p<0.05).

- Cooking loss:** Muscles treated with vitamin E had lower (p<0.05) cooking loss (50.42, 56.98 and 55.89% for LD, SM and BF respectively) as compared with control (C) treatment (53.00, 57.87 and 57.08 % respectively) (Table 2). This result is probably due to vitamin E restricted disruption of cell membranes by reacting free radical, and consequently preventing oxidative deterioration and improve the ability of meat to retain moisture and hold sarcoplasmic components to be retained in muscle cell and thereby resulted in more moisture retention and increased WHC of muscle cell (18). LD, SM and BF muscles injected with distilled water (W) had higher (p<0.05) cooking loss (as compared with C treatment and other treatment (Table 2). As expected injection regardless of the composition of the solution led to increase the exudative loss and a greater loss of moisture during cooking (19). Muscles injected with A<sub>1</sub> and A<sub>2</sub> resulted in a lower (p<0.05) cooking loss [(48.90, 51.85 and 51.02%) and (48.12, 50.96 and 50.05% for LD, SM and BF respectively)] compared with the control and other treatments (E, E+W and W) (Table 2). This result may be due to the impact of kiwifruit juice enzyme on muscle protein and their ability to bind water on the proteins. The results in Table (2) showed also that LD, SM and BF muscles for E+A<sub>1</sub> and E+A<sub>2</sub> recorded lower (p<0.05) cooking loss [(46.58, 49.19 and 48.89%) and (45.40, 47.90 and 47.06%) respectively] compared with the control and other treatments. This may be due to the synergistical action between vitamin E (cell membranes are protected from disruption) and kiwifruit protease enzyme on muscle proteins and their action on the muscle fibre degradation for supplying charged sites available for water binding with protein (16), thereby reduced cooking loss., LD muscles had the lowest (p<0.05) cooking loss as compared with SM and BF muscles, this may be due to high moisture content in LD muscles (Table 2).

**Table (2) Effect of vitamin E kiwifruit juice extract, distilled water pre-rigor injection treatments and their combinations on cooking loss percentage in LD, SM and BF muscles of Karadi sheep (Means  $\pm$  S.E)**

Treat. Type of M.	C	E	W	E+W	A <sub>1</sub>	A <sub>2</sub>	E+A <sub>1</sub>	E+A <sub>2</sub>
LD	53.00 $\pm$ 0.08 a A	50.42 $\pm$ 0.04 b A	54.23 $\pm$ 0.04 c A	52.17 $\pm$ 0.07 d A	48.90 $\pm$ 0.04 e A	48.12 $\pm$ 0.05 f A	46.58 $\pm$ 0.05 g A	45.40 $\pm$ 0.04 h A
SM	57.87 $\pm$ 0.06 a B	56.98 $\pm$ 0.05 b B	59.15 $\pm$ 0.06 c B	56.98 $\pm$ 0.06 b B	51.85 $\pm$ 0.07 d B	50.96 $\pm$ 0.06 e B	49.19 $\pm$ 0.08 f B	47.90 $\pm$ 0.04 g B
BF	57.08 $\pm$ 0.07 a C	55.89 $\pm$ 0.08 b C	58.04 $\pm$ 0.06 c C	56.68 $\pm$ 0.51 d B	51.02 $\pm$ 0.05 e C	50.05 $\pm$ 0.05 f C	48.89 $\pm$ 0.04 g B	47.06 $\pm$ 0.07 h C

C: control, E: vit E, W: water, E+w,

A<sub>1</sub> and A<sub>2</sub>: crude kiwifruit extract at concentrations 8 and 12 respectively.

E+A<sub>1</sub> and E+A<sub>2</sub>: vit E+A<sub>1</sub> (8%) and vit E+A<sub>2</sub> (12%).

Means having different small letters (abc.) among treatments for each muscle are significantly different (p<0.05).

Means having different capital letters (ABC) among muscles for each treatment are significantly different (p<0.05).

#### - Lipid oxidation indicator:

- TBA Value:** The results in Table (3) revealed that dietary vitamin E supplementation to sheep didn't allow TBA to increase (p<0.05) which were 1.04, 1.23 and 1.33 mg malon aldehyde (MDA)/kg muscle for the supplemented group with vit. E while were 1.62, 1.92 and 2.14 mg MDA/Kg muscle for the control group in LD, SM and BF muscles, respectively (Table 3). This result may be due to the action of vitamin E as antioxidant by reacting with free radicals (20) producing from oxidative reactions initiated in the phospholipid-rich membranes of meat (21, 22). Muscles injected with distilled water (W) resulted in the highest (p<0.05) TBA values, which were 1.85, 2.27 and 2.48 mg MDA/kg muscle for LD, SM and BF muscles respectively followed by E+W treatment compared with the control (C) and other treatments. The injection process with distilled water led to increase the exudative loss, caused adverse effect on muscle cell membranes stability, this effect is presumably due to an increase of lipid oxidation in these membranes by action of lipolysis enzymes such as lipase and phospholipase (23). The results in Table (3) showed that LD, SM and BF muscles that muscles injected with A<sub>1</sub> and A<sub>2</sub> treatments had lower (p<0.05) TBA values than control, W and E+W treatments, but was less efficient in retarding oxidative process than vitamin E (Table 3). It was also observed that crude kiwifruit extract was less effective in retarding lipid oxidation than vitamin E. This result may be due to the loss of some natural endogenous antioxidants during preparation of extract or due to its natural antioxidant content (24, 25). The results in Table (3) revealed that LD, SM and BF muscles from sheep supplemented with vitamin E then injected with crude juice extract at concentration of 12% (E+A<sub>2</sub>) had lower TBA values, followed by vitamin E supplemented sheep and injected with crude juice extract at concentration of 8% (E+A<sub>1</sub>) as compared with the control and other treatments. This result showed that muscles from sheep supplemented with vitamin E (presence of vitamin E within muscles) and injected with crude juice extract helped to inhibit the enzyme polyphenol oxidase that determines an oxidation of the endogenous antioxidant in crude kiwifruit juice extract. Previous reports showed that addition of vitamin E with infusion kiwifruit juice solution, played key role in lowering TBA values (26). Previous investigations also showed that lipid oxidation reduced by dietary supplementation vitamin E in lamb meats (27). The result in Table (3) indicated the presence of significant differences (p<0.05) in TBA values among muscles. LD muscles had lower (p<0.05) TBA values than both SM and BF muscles. This result may be due to the lower fat content in LD muscles than both SM and BF muscles. Furthermore, oxidative process is depend on muscle type, therefore, that red fibres (SM and BF) were more suitable locations for lipid oxidation than the white fibre (LD muscles) (28).

**Table (3) Effect of dietary vitamin E, kiwifruit juice extract, distilled water pre-rigor injection treatments and their combinations on thiobarbituric acid (TBA) (mg malon aldehyde/kg muscle) in LD, SM and BF muscles of Karadi sheep (Means  $\pm$ S.E)**

Treat. Type of M.	C	E	W	E+W	A <sub>1</sub>	A <sub>2</sub>	E+A <sub>1</sub>	E+A <sub>2</sub>
LD	1.62 $\pm$ 0.01 a A	1.04 $\pm$ 0.01 b A	1.85 $\pm$ 0.01 c A	1.53 $\pm$ 0.01 d A	1.25 $\pm$ 0.01 e A	1.14 $\pm$ 0.02 f A	0.84 $\pm$ 0.01 g A	0.68 $\pm$ 0.02 h A
SM	1.92 $\pm$ 0.01 a C	1.23 $\pm$ 0.01 b C	2.27 $\pm$ 0.01 c C	1.75 $\pm$ 0.01 d C	1.32 $\pm$ 0.01 e C	1.21 $\pm$ 0.01 b C	0.96 $\pm$ 0.01 f C	0.79 $\pm$ 0.01 g C
BF	2.14 $\pm$ 0.01 a B	1.33 $\pm$ 0.01 b B	2.48 $\pm$ 0.02 c B	2.06 $\pm$ 0.02 d B	1.43 $\pm$ 0.01 e B	1.32 $\pm$ 0.01 b B	1.09 $\pm$ 0.03 f B	0.92 $\pm$ 0.01 g B

C: control, E: vit E, W: water, E+w,

A<sub>1</sub> and A<sub>2</sub>: crude kiwifruit extract at concentrations 8 and 12 respectively.

E+A<sub>1</sub> and E+A<sub>2</sub>: vit E+A<sub>1</sub> (8%) and vit E+A<sub>2</sub> (12%).

Means having different small letters (abc..) among treatments for each muscle are significantly different ( $p < 0.05$ ).

Means having different capital letters (ABC) among muscles for each treatment are significantly different ( $p < 0.05$ ).

- Sensory evaluation:** The results presented in Table (4) showed that E+A<sub>2</sub>, E+A<sub>1</sub> and E treatments had significantly ( $p < 0.05$ ) higher scores for colour of LD and BF muscle, A<sub>1</sub> and A<sub>2</sub> treatments, had a moderate colour scores for LD, SM and BF muscles compared with scores of the control treatment in LD, SM and BF muscles. Water treatment (W) had the lowest values for the colour of the LD, SM and BF muscles. LD, SM and BF muscles of the E+A<sub>2</sub> and E+A<sub>1</sub> treatments were significantly ( $p < 0.05$ ) more desirable and preferred color than the control (C), W and E+W treatments (Table 4). Colour and flavour and aroma of muscles of treatments E+A<sub>2</sub> and E+A<sub>1</sub> were obtained higher score than the A<sub>2</sub> and A<sub>1</sub> alone, and similar results were found for TBA values (Table 3). These results may be due to the presence of vitamin E within muscles as well as kiwifruit extract contains amount ascorbic acid, which, acted stronger synergism to retard lipid oxidation and maintains the desired meat colour in muscles (29, 30). The results in Table (4) showed that E+A<sub>2</sub>, E+A<sub>1</sub> and A<sub>2</sub> treatments had significantly ( $p < 0.05$ ) higher tenderness and juiciness score in LD, SM and BF muscle as compared with the values of the C, W and E treatments. The clear improvement in meat tenderness and juiciness of the treated muscles with E+A<sub>2</sub>, E+A<sub>1</sub> and A<sub>2</sub> treatments may be due to the proteolytic activity of the kiwifruit protease actinidin in degradation of muscle myofibrillar proteins (31). it could possibly be due to calcium in the kiwifruit, which contains calcium at 26 mg/100g fresh weight, as well as the improvement in meat juiciness in these treatments, it may be due to the decrease cooking loss, increased protein solubility and moisture retention (32). The results in Table (4) demonstrated that the highest scores were obtained for all sensory attributes at E+A<sub>2</sub> treatment for all muscles. This result revealed that LD muscles were more acceptable in the sensory evaluation, particularly tenderness and juiciness attributes than SM and BF muscles, this may be due to the considerable differences in the myofibrils and collagen content of these muscles, thus LD muscle had a lower collagen and higher water holding capacity (1, 33).

**Table (4) Effect of dietary vitamin E supplementation, different concentrations of kiwifruit juice extract, distilled water pre-rigor injection treatments and their combinations on sensory evaluation score in LD, SM and BF muscles of Karadi sheep (Means  $\pm$  S.E)**

Treat.	Colour			Flavor & Aroma			Tenderness			Juiciness			Overall acceptability		
	LD	SM	BF	LD	SM	BF	LD	SM	BF	LD	SM	BF	LD	SM	BF
<b>C</b>	2.75 $\pm 0.25$ aA	2.50 $\pm 0.29$ ac A	2.75 $\pm 0.25$ ac A	2.50 $\pm 0.29$ a A	2.25 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.50 $\pm 0.29$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.50 $\pm 0.29$ ab A	2.25 $\pm 0.25$ a A	2.75 $\pm 0.25$ abc A
<b>E</b>	3.75 $\pm 0.25$ d A	3.25 $\pm 0.25$ b A	3.50 $\pm 0.29$ bcd A	4.00 $\pm 0.00$ bc A	3.50 $\pm 0.29$ b A	3.75 $\pm 0.25$ b A	3.00 $\pm 0.00$ ab A	2.50 $\pm 0.29$ ab A	2.75 $\pm 0.25$ ab A	3.00 $\pm 0.00$ ab A	2.50 $\pm 0.29$ ab A	2.75 $\pm 0.25$ ab A	3.00 $\pm 0.00$ ac A	2.50 $\pm 0.29$ a A	2.75 $\pm 0.25$ abc A
<b>W</b>	2.50 $\pm 0.29$ aA	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.25 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	3.00 $\pm 0.00$ ab A	2.25 $\pm 0.25$ a B	2.75 $\pm 0.25$ ab AB	2.25 $\pm 0.25$ b A	2.25 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A
<b>E+W</b>	3.00 $\pm 0.00$ ac A	2.50 $\pm 0.29$ ac A	2.75 $\pm 0.25$ ac A	2.75 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.75 $\pm 0.25$ a A	2.25 $\pm 0.25$ a A	2.50 $\pm 0.29$ a A	2.75 $\pm 0.25$ a A	2.50 $\pm 0.29$ ab A	2.75 $\pm 0.25$ ab A	2.50 $\pm 0.29$ ab A	2.50 $\pm 0.29$ a A	2.50 $\pm 0.29$ ab A
<b>A<sub>1</sub></b>	3.50 $\pm 0.29$ bc A	3.00 $\pm 0.00$ bcd A	3.25 $\pm 0.25$ cd A	3.50 $\pm 0.29$ b A	3.00 $\pm 0.00$ b A	3.25 $\pm 0.25$ b A	3.50 $\pm 0.29$ bc A	2.50 $\pm 0.29$ a B	3.00 $\pm 0.00$ abc AB	3.50 $\pm 0.29$ bc A	2.75 $\pm 0.25$ abc B	3.00 $\pm 0.00$ ab AB	3.25 $\pm 0.25$ c A	2.75 $\pm 0.25$ ab A	3.00 $\pm 0.00$ bcd A
<b>A<sub>2</sub></b>	3.50 $\pm 0.29$ bc A	3.25 $\pm 0.25$ d A	3.50 $\pm 0.29$ d A	3.50 $\pm 0.29$ b A	3.00 $\pm 0.00$ b A	3.25 $\pm 0.25$ b A	4.00 $\pm 0.00$ cd A	3.00 $\pm 0.00$ bc B	3.25 $\pm 0.25$ bc B	3.75 $\pm 0.25$ cd A	3.00 $\pm 0.00$ bc B	3.00 $\pm 0.00$ ab B	3.50 $\pm 0.29$ c A	3.25 $\pm 0.25$ b A	3.25 $\pm 0.25$ cd A
<b>E+A<sub>1</sub></b>	4.00 $\pm 0.00$ bd A	3.25 $\pm 0.25$ d B	3.50 $\pm 0.29$ d AB	4.25 $\pm 0.25$ c A	3.25 $\pm 0.25$ b B	3.50 $\pm 0.29$ b AB	4.50 $\pm 0.29$ d A	3.25 $\pm 0.25$ c B	3.25 $\pm 0.25$ bc B	4.00 $\pm 0.00$ cd A	3.25 $\pm 0.25$ c B	3.25 $\pm 0.25$ b B	4.25 $\pm 0.25$ d A	3.25 $\pm 0.25$ b B	3.50 $\pm 0.29$ d AB
<b>E+A<sub>2</sub></b>	4.25 $\pm 0.25$ d A	3.25 $\pm 0.25$ d B	3.50 $\pm 0.29$ d AB	4.25 $\pm 0.25$ c A	3.25 $\pm 0.25$ b B	3.50 $\pm 0.29$ b AB	4.50 $\pm 0.29$ d A	3.50 $\pm 0.29$ c B	3.50 $\pm 0.29$ c B	4.25 $\pm 0.25$ d A	3.25 $\pm 0.25$ c B	3.25 $\pm 0.25$ b B	4.50 $\pm 0.29$ d A	3.25 $\pm 0.25$ b B	3.50 $\pm 0.29$ d B

Means having different small letters (abc..) among treatments for each muscle are significantly different ( $p < 0.05$ ).

Means having different capital letters (ABC) among muscles for each treatment are significantly different ( $p < 0.05$ ).

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