

# Effect of Feeding Regime on Meat Quality of Elk Deer Loin during Aging

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**Abstract** The study aimed to investigate the effects of feeding regimes on chemical composition, meat quality, and fatty acid composition of elk deer loin (EDL) while aging at 4°C. Sixteen 3-year-old elk hinds were randomly assigned to one of two dietary treatments: pasture grazing (PG) and barn feeding (BF). While eight elks grazed on pastures with supplementary feed of 1.0% of body weight (PG), the remaining eight elks were fed 1.0% of concentrate with hay *ad libitum* for 5 months in a barn (BF). After slaughtering, EDL was dissected and aged for 56 days at 4°C under vacuum packaging. Proximate composition, physicochemical characteristics, and fatty acid composition of EDL were determined thereafter. Proximate composition of EDL showed no significant difference between feeding regimes and across aging period. The pH values of EDL increased with increase of storage days. Drip loss increased by day 14, compared to that on the first day, and shear force decreased during aging. Water holding capacity and cooking loss were not significantly affected by feeding regime or aging period. Lightness (L\*) and redness (a\*) were decreased on day 56 compared to the initial day. Yellowness (b\*) of EDL decreased with increase of aging time. The  $\alpha$ -linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid content was higher in PG treatment and increased with aging duration. Both feeding regimes and aging were found to affect meat quality and fatty acid profiles.

**Keywords:** elk deer meat, feeding regime, aging, meat quality

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## 1. Introduction

Safety of animal-derived food and livestock diseases raise concerns about the pros and cons of meat production and consumption. Moreover, consumers are increasingly getting aware about the environment and conditions of meat production, preferring eco-friendly animal-origin food (free range products) and low input system [1]. Venison has a positive impact, for being a natural, "organic," and safe meat product, since the animal is allowed to graze naturally, without fattening feed additives or high-calory fodder, and has limited contact with animals using pharmaceuticals. It is considered a healthy meat source owing to its taste, low fat content, and high levels of polyunsaturated fatty acids (PUFAs) and minerals [2,3].

Many studies have shown feeding regime to modify the meat quality. Especially, studies comparing the effects of pasture and grain-based feeding have demonstrated

feeding process to play a crucial role in the quality of meat from ruminants. Meat from pasture-raised ruminants have higher n-3 PUFA, with higher oxidative stability due to higher antioxidant concentration [3,4,5,6]. Moreover, meat from grass-fed animals are characterized by longer shelf life during storage and higher a\* value compared to grain- and hay-fed animals [7].

Meat aging under vacuum packaging can also influence meat quality, although that is a common method in meat industry worldwide. The biochemical changes that modulate meat quality dictate the shelf life during postmortem aging [8]. Tenderness is the major trait that needs to be improved during aging. Venison is considered to be less tender than beef due to its low fat content. Tenderness (meat quality) of venison can be improved by aging; however, little is known regarding the influence of feeding regimes and aging time on elk meat quality with microbial status and oxidation.

The present study aimed to investigate the effects of feeding regimes and aging time at 4°C on elk meat quality and shelf life during aging.

## 2. Materials and Methods

### 2.1. Animal Feed and Sample Preparation

Sixteen 3-year-old elk hinds were stratified by weight and randomly assigned to one of the two feeding regimes (pasture grazing (PG) and barn feeding (BF)), and raised for 5 months from May to September 2019 at the Animal Genetic Resources Research Center, National Institute of Animal Science (NIAS), Korea. Eight elks were allowed to graze in pasture *ad libitum* with supplements, whereas the other eight were fed 1.0% concentrates/body weight (B.W.) and hay *ad libitum*, during the feeding period, the feed being a major roughage source for deer in Korea. Nutritional content of the concentrates and hay included 85.9% dry matter (DM) and 18.8% crude protein, and 81.2% DM and 9.9% crude protein, respectively. Pasture samples were collected thrice from six different locations within each pasture during the experimental periods. Pastures had predominantly tall fescue with an average nutritional value of 22% DM, 10.8% crude protein, and average forage productivity of  $1,569 \pm 720$  kg DM/ha. Animals had free access to freshwater and mineralized salt blocks. All animal-based procedures were performed in accordance with the standard guidelines for the Care and Use of Experimental Animals provided by the National Institute of Animal Science, RDA, Korea (permit number NIAS-2014-088). After the feeding trial, all animals were slaughtered using standard NIAS procedures. Carcasses were stored in cold room (4°C) for 24 h prior to dissection. After boning, the excised elk deer loin (EDL) samples were vacuum-packed and aged for 56 days at 4°C. Every 14 days (day 0, day 14, day 28, day 42, and day 56), the aged deer loin was used for analysis.

### 2.2. Physicochemical Analysis

Proximate composition of elk deer loin was determined using the modified methods of Association of Official Analytical Chemists (AOAC) [9]. To determine the dry matter content, 3 g of minced meat was dried to a constant weight at 105°C. Crude protein content was analyzed using Kjeldahl method while crude lipid was analyzed by Soxhlet extraction with diethyl ether as the solvent. The ash content was measured by incineration at 550°C to a constant weight.

pH values of the samples were measured by blending 10 g of a sample with 90 mL distilled water for 60 s in a homogenizer (PolyTron PT-2500 E, Kinematica, Lucerne, Switzerland), followed by pH measurement using a pH meter (Orion 230A, Thermo Fisher Scientific, Waltham, MA, USA).

Water holding capacity (WHC) was analyzed using the modified method described by Kristensen and Purslow [10]. A sample (0.5 g) of minced meat was heated for 20 min at 80°C in a water bath and cooled to room temperature (23°C). After cooling, the samples were centrifuged at  $2,000 \times g$  for 20 min and total moisture was measured. The values of WHC were calculated by the following equation.  $WHC (\%) = [(total\ water\ content - separated\ water\ content) / total\ water\ contents] \times 100$ .

For cooking loss, samples in vacuum-sealed bags were weighed and cooked for 45 min at 80°C in a water bath. Thereafter, samples were cooled at  $23 \pm 2^\circ C$  (room temperature) until the temperature at the center of the meat reached 75°C, and then re-weighed. Cooking loss was calculated based on the difference between the weights of raw and final cooked meat as per the following equation:  $Cooking\ loss (\%) = [(weight\ before\ cooking - weight\ after\ cooking) / weight\ before\ cooking] \times 100$ .

Color measurements were performed in triplicate with a Minolta Chroma Meter (Model CR-300, Minolta Co., Osaka, Japan), and the Commission Internationale de l'Eclairage (CIE) color values for CIE L\*, CIE a\*, and CIE b\* were determined. The chromameter was standardized using a white calibration plate ( $Y = 93.6$ ,  $x = 0.3134$ ,  $y = 0.3194$ ).

Shear force values per core were obtained for each animal from  $1 \times 2 \times 2$  cm loin meat samples that had been cooked in water bath till an internal temperature of 75°C. The muscle samples were cut perpendicular to the muscle fiber and assessed by a texture analyzer (TA 1; Lloyd Instruments, Berwyn, PA, USA) with a V-shaped blade. Test settings were: 50 kg load cell, 50 mm/min trigger speed, 50 mm/min test speed, and 10 gf trigger force.

### 2.3. Aerobic Plate Counts (APC) and *E. Coli*/Coliforms

To determine the microbes, 10 g loin sample was homogenized with 90 mL peptone water in stomacher bag (Bag Mixer 400, INTERSCIENCE, St. Nom, France). After serial dilution, 1 mL of the diluent was loaded on to Petrifilms (3M Microbiology, St. Paul, MN, USA) for APC and *E. coli*/coliform count. The films for APC and *E. coli*/coliforms were incubated at appropriate temperature as per the manufacturer's instructions.

### 2.4. Volatile Basic Nitrogen (VBN)

Ten grams of meat sample and 50 mL of distilled water were stirred for 30 min, and then filtered through a filter paper (Whatman No. 1). The sample filtrate and 0.01 N H<sub>2</sub>SO<sub>4</sub> were loaded onto Conway unit and incubated for 1 h at 25°C. Thereafter, 10 µL of the Brunswick indicator was added to the inner chamber of the Conway unit and titrated with 0.01 N NaOH. The VBN value was calculated using the equation mentioned by Kim et al. [11] and expressed as mg/100 g.

### 2.5. 2-Thiobarbituric Acid Reactive Substance (TBARS)

The TBARS value was measured according to the method of Al-Hijazeen et al. [12] it was calculated using the following equation:

$$TBARS \ (mg\ MDA / kg) = \left( \frac{Absorbance\ of\ sample}{-Absorbance\ of\ blank\ sample} \right) \times 5.88$$

and expressed as mg of malondialdehyde (MDA) per kg of meat.

## 2.6. Fatty Acid Composition

Total fat for fatty acid analysis was extracted using chloroform/methanol (2:1, v/v) according to the method of Folch et al. [13] Lipids were extracted in chloroform/methanol (2:1), with 10% butyl hydroxyl toluene as an antioxidant. An aliquot of the total lipid extract was methylated as described by Morrison and Smith [14]. Fatty acid methyl ester was analyzed using a gas chromatograph (6890N, Agilent Technologies, Palo Alto, CA, USA), equipped with a CP-Sil 88 capillary column (100 m × 0.25 mm × 0.20 μm, Agilent Technologies, Palo Alto, CA, USA). Helium was used as the carrier gas at a linear flow of 1 mL/min. The oven temperature was initially held at 200°C for 10 min, and then increased at 3°C/min up to 250°C for 5 min. The injection port was at 250°C and the detector was maintained at 300°C. Results were expressed as percentages based on the total peak area.

## 2.7. Statistical Analysis

The response variables were analyzed using two-way ANOVA with the general linear model procedure of SPSS 18 (SPSS, Inc., Chicago, IL, USA). The model included terms for feeding system, storage duration, and the interaction between feeding system and storage duration. When significant difference was found, Duncan's multiple range test was performed at  $p < 0.05$ .

## 3. Results

### 3.1. Physicochemical Characteristics of Deer Loin Meat

Moisture, crude protein, ether extract, and crude ash composition of EDL is shown in Table 1. The approximate compositions were not affected by feeding regimes and aging times. The physicochemical properties of EDL during aging are shown in Table 2. pH was significantly affected by aging time while no significant effect was seen due to feeding regime. Cooking loss and WHC were not affected by either feeding regime or aging time. Interestingly, shear force value of EDL was significantly affected by both feeding system and aging time ( $p < 0.05$ ); the shear force was significantly reduced with increase in aging time. EDL of barn-fed animals showed lower shear force than that of grazing animal, on the first day of aging. However, no further influence of feeding regime and aging time was seen on the same.

Effects of feeding regimes on meat color of EDL during aging are presented in Table 3. Although the lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) of EDL were not affected by feeding regimes, the color values were significantly affected by aging time ( $p < 0.05$ ). The  $L^*$  and  $a^*$  values were significantly reduced, whereas the  $b^*$  value was significantly increased, with aging time ( $p < 0.05$ ).

**Table 1. Effect of Feeding Regime on the Proximate Composition of Elk Deer Loin during Ageing**

Proximate composition (%)	Treatment										SEM1)	Significance2)		
	Barn-feeding					Pasture grazing						F	A	F × A
	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56				
Moisture	73.7	73.8	73.5	73.4	73.5	73.2	73.2	73.2	73.2	73.3	0.081	*3)	ns	ns
Crude protein	25.3	25.1	25.0	24.9	25.0	25.3	25.3	25.2	24.8	25.3	0.055	ns	ns	ns
Crude fat	0.80	0.79	0.81	0.81	0.82	0.78	0.79	0.80	0.81	0.83	0.005	ns	ns	ns
Crude ash	1.16	1.15	1.19	1.19	1.20	1.20	1.16	1.19	1.18	1.18	0.009	ns	ns	ns

<sup>1)</sup> SEM; Standard error of the means.

<sup>2)</sup> Significance: F, feeding system; A, aging time; F × A, interaction between F and A.

<sup>3)</sup> ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Table 2. Effect of Feeding Regime on the Physicochemical Characteristics of Elk Deer Loin during Ageing**

Item	Treatment										SEM1)	Significance2)		
	Barn-feeding					Pasture grazing						F	A	F × A
	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56				
pH	5.39a	5.55bc	5.56bc	5.57bc	5.59bc	5.36a	5.51b	5.54bc	5.57bc	5.61c	0.017	0.515	<0.05	0.824
WHC3 (%)	48.7	49.9	47.1	48.6	49.4	49.2	50.6	48.8	49.8	48.8	0.465	0.489	0.749	0.968
Cooking loss (%)	31.1	31.7	32.9	32.8	33.9	31.5	32.1	30.8	30.3	32.9	0.360	0.214	0.402	0.606
Shear force (kgf)	9.37e	7.44 cd	6.24bc	5.14ab	4.59a	10.8f	8.64 de	7.31 cd	5.41ab	4.52a	0.398	<0.05	<0.05	0.426

<sup>a-f</sup> Means within the same row with different letters are significantly different ( $p < 0.05$ ).

<sup>1)</sup> SEM; Standard error of the means.

<sup>2)</sup> Significance: F, feeding system; A, aging time; F × A, interaction.

<sup>3)</sup> WHC; water holding capacity.

<sup>4)</sup> ns, not significant; \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ .

**Table 3. Effect of Feeding Regime on the Meat Color of Elk Deer Loin during Ageing**

Item	Treatment										SEM1)	Significance2)		
	Barn-feeding					Pasture grazing						F	A	F × A
	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56				
CIE $L^*$	30.6bc	30.8c	30.9c	30.6bc	29.8a	30.7c	30.7c	30.7c	30.1ab	29.7a	0.087	0.215	<0.05	0.528
CIE $a^*$	16.2c	15.9bc	15.5ab	15.3a	15.5ab	15.8abc	15.7abc	15.6ab	15.4a	15.4ab	0.069	0.153	<0.05	0.567
CIE $b^*$	6.68a	7.51b	8.16c	8.68d	8.95d	6.80a	7.33b	8.04c	8.78d	8.87d	0.154	0.635	<0.05	0.576

<sup>a-d</sup> Means within the same row with different letters are significantly different ( $p < 0.05$ ).

<sup>1)</sup> SEM; Standard error of the means.

<sup>2)</sup> Significance: F, feeding system; A, aging time; F × A, interaction.

### 3.2. Microbial Counts, VBN, and TBARS of Deer Loin Meat

Microbial counts (APC, *E. coli*, and coliforms), protein decomposition (VBN), and lipid oxidation (TBARS) of EDL during aging are shown in Table 4. Aerobic plate counts of EDL were significantly affected by feeding regime and aging time (p = 0.000). Especially, total microbes in EDL from grazing animals were significantly higher than those in EDL from barn-fed animals after 14 days of aging. However, effect of feeding regime and aging time on the total microbial numbers has not been shown. *E. coli* and

coliforms were not detected until day 42 of aging.

VBN of EDL was significantly increased during aging in both feeding regimes. At day 56, the VBN values for barn-fed and pasture-raised animals were 20.38 and 19.43 mg/100 g, respectively. It significantly interacted with both feeding regime and aging time. TBARS of EDL significantly increased with aging time. It was in the range of 0.13-0.68 mg MDA/kg meat until day 28; however, it increased dramatically up to 2.32-2.80 mg MDA/kg meat at days 42 and 56. Feeding regime significantly affected TBARS of EDL, although no significant interaction was found between feeding regime and aging time.

**Table 4. Effect of Feeding Regime on the Microbes, VBN, and TBARS Value of Elk Deer Loin During Ageing**

Item	Treatment										SEM1)	Significance2)		
	Barn-feeding					Pasture grazing						F	A	F × A
	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56				
Aerobic plate counts (Log CFU/g)	2.16a	3.89c	5.17e	6.07g	6.70i	2.15a	3.59b	4.91d	5.66f	6.31h	0.291	0.000	0.000	0.109
<i>E.coli</i> (Log CFU/g)	nd3)	nd	nd	nd	0.86b	nd	nd	nd	nd	0.67b	0.065	0.606	0.000	0.891
Coliforms (Log CFU/g)	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-
VBN (mg/100 g)	9.35a	10.04c	10.87d	14.79f	20.38h	9.50ab	9.89bc	10.72d	14.22e	19.43g	0.730	0.001	0.000	0.007
TBARS (mg MDA/kg)	0.13g	0.38f	0.68e	2.44c	2.80a	0.13g	0.34f	0.61e	2.32d	2.71b	0.031	0.005	<.0001	0.3568

<sup>a-i</sup> Means within the same row with different letters are significantly different (p < 0.05).

<sup>1)</sup> SEM: Standard error of the means.

<sup>2)</sup> Significance: F, feeding system; A, aging time; F × A, interaction.

<sup>3)</sup> nd: not detected.

**Table 5. Effect of Feeding Regime on the Fatty Acid Composition of Elk Deer Loin During Ageing**

Item	Treatment										SEM1)	Significance2)		
	Barn-feeding					Pasture grazing						F	A	F × A
	Day 0	Day 14	Day 28	Day 42	Day 56	Day 0	Day 14	Day 28	Day 42	Day 56				
C14:0 (Myristic acid)	2.87ab	2.93ab	2.83a	2.91ab	3.94c	2.77a	3.09ab	3.47abc	3.59bc	3.04ab	0.090	0.498	0.065	<0.050
C16:0 (Palmitic acid)	23.0	23.9	23.5	23.4	25.5	23.2	24.0	24.6	23.5	25.0	0.262	0.732	0.155	0.906
C16:1n7 (Palmitoleic acid)	5.83	6.20	6.24	6.34	5.86	5.53	6.52	5.84	5.43	5.55	0.130	0.247	0.509	0.716
C18:0 (Stearic acid)	14.8	14.5	13.9	13.5	14.8	15.0	14.3	14.2	14.7	14.7	0.188	0.504	0.569	0.799
C18:1n9 (Oleic acid)	11.6abc	12.3cd	11.7abc	12.4cd	13.0d	10.8ab	10.6a	12.0bcd	11.5abc	11.6abc	0.160	<0.050	0.078	0.165
C18:1n7 (Vaccenic acid)	7.45a	8.23ab	8.78b	7.73ab	7.44a	7.09a	8.99b	7.09a	6.93a	7.96ab	0.163	0.222	<0.050	<0.050
C18:2n6 (Linoleic acid)	17.6	16.5	16.7	17.3	15.3	18.1	16.3	16.7	17.2	16.4	0.259	0.627	0.189	0.925
C18:3n6 (γ-Linolenic acid)	0.18a	0.16a	0.16a	0.20ab	0.24bc	0.20ab	0.17a	0.31de	0.26cd	0.35e	0.012	<0.050	<0.050	<0.050
C18:3n3 (Linolenic acid)	1.39bc	1.36bc	1.49abc	1.33b	1.02a	1.80e	1.70de	1.5abc	1.63cde	1.30b	0.046	<0.050	<0.050	0.234
C20:1n9 (Eicosenoic acid)	1.11	1.03	0.86	1.07	1.04	0.89	0.87	0.84	1.00	1.18	0.037	0.387	0.280	0.587
C20:4n6 (Arachidonic acid)	10.76	9.73	10.43	10.46	9.10	10.50	9.52	9.39	10.08	9.41	0.209	0.482	0.342	0.912
C20:5n3 (Eicosapentaenoic acid)	0.68ab	0.63ab	0.63ab	0.64ab	0.54a	1.01d	0.89cd	0.94d	0.92cd	0.78bc	0.031	<0.050	<0.050	0.881
C22:4n6 (Adrenic acid)	0.91bc	0.87abc	0.98c	0.84abc	0.69a	0.77ab	0.76ab	0.70a	0.73ab	0.70a	0.023	<0.050	0.138	0.236
C22:6n3 (Docosahexaenoic acid)	1.89abc	1.73ab	1.85ab	1.94abc	1.61a	2.38de	2.26cde	2.45de	2.61e	2.13bcd	0.067	<0.050	<0.050	0.957
SFA3)	40.7ab	41.4ab	40.2ab	39.8a	44.2b	41.0ab	41.4ab	42.3ab	41.7ab	42.7ab	0.394	0.483	0.208	0.559
USFA	59.4ab	58.7ab	59.8ab	60.2b	55.8a	59.1ab	58.6ab	57.7ab	58.3ab	57.4ab	0.394	0.478	0.209	0.562
MUFA	33.4ab	30.9ab	32.3ab	32.7ab	28.5a	34.7b	31.6ab	32.0ab	33.5ab	31.1ab	0.501	0.307	0.089	0.909
PUFA	26.0abc	27.7c	27.6bc	27.5bc	27.3bc	24.3a	27.0bc	25.7abc	24.9ab	26.3abc	0.300	<0.050	0.112	0.789
n6: n3	7.45c	7.33c	7.15bc	7.42c	7.97c	5.68a	5.52a	5.53a	5.51a	6.40ab	0.187	<0.050	<0.050	0.972

<sup>a-e</sup> Means within the same row with different letters are significantly different (p < 0.05).

<sup>1)</sup> SEM: Standard error of the means.

<sup>2)</sup> Significance: F, feeding system; A, aging time; F × A, interaction.

<sup>3)</sup> SFA, saturated fatty acid; USFA, unsaturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

### 3.3. Fatty Acid Composition

The effect of feeding regimes on fatty acid composition of deer loin during aging is presented in Table 5. Palmitic, palmitoleic, and stearic acids did not get affected by feeding regime or aging time. Myristic acid of deer loin in BF was higher than that in PG on day 56. Linoleic acid was not affected by either of feeding regime and aging time. The oleic acid of deer loin in PG system was lower than that in BF system on days 14 and 56. The  $\gamma$ -linolenic acid content increased with increase in aging time, whereas alpha-linolenic acid (ALA) content decreased during storage in both BF and PG systems. The  $\gamma$ -linolenic acid and ALA of deer loin in PG system were higher than those in BF system. The eicosapentaenoic acid (EPA) of deer loin in PG system was higher than that in BF system ( $p < 0.05$ ). Effect of feeding regime and aging was also observed for docosahexaenoic acid (DHA); it was high in PG system on days 0, 14, and 28. Saturated fatty acid (SFA), unsaturated fatty acid (USFA), and monounsaturated fatty acid (MUFA) did not show difference across treatments and across aging times during treatment. The polyunsaturated fatty acid (PUFA) content was higher on days 14 to 56 than on day 0.

## 4. Discussion

### 4.1. Physicochemical Characteristics of Deer Loin

Physicochemical analysis was performed to assess the impact of *ante*- (feeding regimes) and *post*- (aging) mortem factors on meat quality. Neither feeding regime nor aging time affected the chemical composition of venison in the present study. Similar chemical compositions had been noted by other authors in both fallow and red deer [1,15]. Mean values of protein content in venison ranged from 20 to as high as 25% [16], indicating relatively rich protein content of venison. Venison has been demonstrated to be valued by consumers due to their low-fat and protein-rich properties.

pH value is one of the most crucial parameters determining the quality of meat, and normal pH for venison ranges from 5.5 to 5.7 [17]. Similar nutrient status did not affect muscle glycogen stores or pH values in fallow deer [18,19]. In the present study, the pH values of deer loin ranged from 5.36 to 5.61, similar to those reported in fallow deer [19,20] and red deer [21].

In agreement with our findings, previous studies had reported feeding regimes to not affect the physical quality of ruminant species [22,23]. However, the period of aging influenced other physical meat quality attributes. Soriano et al. [24] had reported water loss, in the course of ripening or storage of meat, to be influenced by the proteolytic activity of endogenous muscle enzymes. Farouk et al. [25] had reported proteolytic activity to possibly be related to meat tenderness. Similar relationship between storage and drip loss was observed in pork [26] and beef [27] as well.

Increased meat tenderness had previously been demonstrated to reflect reduced shear force and less

venison juice during storage [20,28], which is similar to our findings. The reduced shear force during aging is due to the proteolytic enzyme activity leading to myofibrillar protein degradation [25,29]. Wiklund et al. [21] had measured calpain activity and reported negative regression between calpain, calpastatin activity, and drip and purge in venison.

Meat color is a crucial factor that affects consumer acceptability of a product and other relevant decisions. Venison color has been demonstrated to be darker than that of other ruminant species [30,31]. Physical activity, including farming systems, rather than feeding regimes, may be attributed to beef color [32]. In contrast, others have reported farming system to not influence meat color in deer [18,19], which is consistent with our current results. Daszkiewicz et al. [33] had reported game meat to be darker than that of farm-raised animals, owing to their high physical activity, thus affecting increased myoglobin content of the muscle. Although the reason for this discrepancy in meat color is still not clear, it might be related to improved nutritional status, such as supplementary feeding. Wild animals show more physical activity than grazing animals due to nutritional deficiency in pasture-feed and their predators. In the present study, the color parameters  $L^*$  and  $a^*$  decreased during aging while  $b^*$  value increased. Wiklund et al. [34] had reported color stability of elk deer loin to be lower than that of beef, since deer meat is vulnerable to oxidative deterioration owing to high levels of pro-oxidants like iron and copper. Oxy-myoglobin is oxidized to brownish metmyoglobin upon consuming muscle oxygen [35]. Mancini and Hunt [32] had reported residual oxygen in package to contribute to oxymyoglobin oxidation to metmyoglobin, thereby indicating higher  $b^*$  values in aged meat. The poor color of venison, during aging, reflects the reducing capacity of metmyoglobin [21].

### 4.2. Shelf Life of Deer Loin Meat

Shelf life of meat is an important quality trait; microbiological property is a key factor to judge the shelf life of meat products [21]. Generally, a critical limit of total aerobic bacteria is set as 7 Log CFU/g. Values greater than or equal to that indicate the meat as unacceptable for human consumption [36]. Different countries regulate meat freshness and microbial shelf life using different limits. APC (*E. coli*) limit of meat in Japan, Australia, European union, and Korea is less than  $5.0 \times 10^5$  CFU/g (negative),  $1.0 \times 10^6$  CFU/g ( $< 1.0 \times 10^3$  CFU/g), 5.0 Log CFU/cm<sup>2</sup> (2.5 Log CFU/cm<sup>2</sup> for cattle, sheep, and horse), and below  $5.0 \times 10^6$  CFU/g or 6.7 Log CFU/g, respectively. In this study, APC was significantly affected by feeding regime and aging times ( $p < 0.05$ ). APC of EDL from barn-fed animals at day 56 was over the regulation limit, indicating the EDL from barn animals to be unacceptable for human consumption, whereas that from pasture-raised animals was within limits. *E. coli* counts affected only the aging time ( $p < 0.05$ ).

During aging, protein decomposition and lipid oxidation deteriorate both meat quality and shelf life. As an indicator of meat freshness, VBN is crucial for protein decomposition and decay. Microorganisms and enzymes

in meat products cause proteolysis, leading to an increase of VBN [37]. Sujiwo et al. [38] had reported the VBN value of meat to be highly correlated with the population of bacteria. Korea Food Code [39] as designated meat with VBN value higher than 20 mg/100 g as spoilt and unacceptable for consumers. Seman et al. [40] had reported vacuum packed venison to be acceptable up to 18 weeks, when stored and distributed at approximately -1°C. In this study, VBN values of venison, after 42 and 56 days of pasture grazing were significantly low compared to that after barn feeding ( $p < 0.001$ ). This may be due to the high amount of polyphenol in pastures that act as an antioxidant and inhibit protein oxidation. The TBARS value is considered to be a lipid oxidation indicator of meat. During meat aging, the TBARS value is increased and indicates rancidity on days 42 and 56 of aging. Meat from pasture-grazing venison showed significantly lower TBARS value than that from concentrate-fed venison after aging for 42 and 56 days. This result may also be due to antioxidants in pastures, including polyphenols that protect lipids from oxidation. Perceptible rancidity was indicated when TBARS value was over 0.8 mg MDA/kg [41]. The TBARS value of venison, in both feeding regimes, until aging day 28, was less than 0.8 mg MDA/kg, hence indicating that lipid rancidity was not perceptible.

### 4.3. Fatty Acid Composition of Deer Loin

The amount and structure of fatty acids (FA) in meat play a crucial role in human health and are influenced by the feeding system [5]. A previous study [28,42] had demonstrated that pasture-fed deer is high in ALA and low in n-6/n-3 ratio compared to concentrate-fed deer, which is in agreement with our study. In this study, n-3 fatty acids, such as  $\gamma$ -linolenic acid and linolenic acid, in pasture-fed venison were higher than in concentrate-fed venison. Wiklund et al. [36] had reported pasture-fed deer to show high content of n-3 long chain fatty acids, such as EPA and DHA, compared to concentrate-fed deer [36,43]. Riediger et al. [44] had reported venison to contain higher proportions of EPA and DHA than other ruminants, thus showing a protective effect against cardiovascular disease. They suggested venison to be a more beneficial meat than other ruminants owing to its higher content of polyunsaturated fatty acids than saturated fatty acids.

## 5. Conclusion

Overall, we found that the grazing pasture effect on high tenderness and n-3 fatty acid composition of EDL as ante-mortem factors. Additionally, according to aging time as post-mortem factors, pasture grazed EDL had longer shelf life than EDL from barn feeding treatment, which extend the shelf life until day 56 of aging. It is suggested that physicochemical quality and fatty acid composition of EDL can be controlled by the change of feeding regime and it affects to final quality during aging time. Further studies are needed to evaluate the effect of feeding regime and aging time on the sensory quality or flavor of EDL.

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## Statement of Competing Interests

The authors have no competing interests.

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