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## Research Article

# Effects of dental age on proximate components, amino acid and fatty acid profiles of indigenous beef

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### Abstract

The study was conducted to determine how the proximate composition, amino acid and fatty acid profiles of beef of indigenous cattle are affected by dental age. This experiment was conducted with five (5) treatments (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, and T<sub>5</sub>) where T<sub>1</sub> = 0 Permanent incisors, T<sub>2</sub> = 2 Permanent incisors, T<sub>3</sub> = 4 Permanent incisors, T<sub>4</sub> = 6 Permanent incisors, T<sub>5</sub> = 8 Permanent incisors having ten (10) replications. This study has shown that the highest CP was 84.91% having the T<sub>5</sub> while the lowest figure was 72.94% in T<sub>4</sub>. T<sub>1</sub> had the lowest 3.34% EE content whereas T<sub>5</sub> had the highest 4.15%. Essential amino acids (g/100g of meat) were highest at 41.51 in T<sub>5</sub> and lowest at 37.79 in T<sub>1</sub>. The lowest total saturated fatty acid (SFA) in *Longissimus dorsi* was 46.25% in T<sub>5</sub> and the highest was 51.89% in T<sub>1</sub>. The total monounsaturated fatty acid (MUFA) proportion of 66.87% in T<sub>2</sub> was the highest and T<sub>5</sub> had the lowest proportion of 52.07%. The lowest total polyunsaturated fatty acid (PUFA) was 1.52% in T<sub>1</sub> and the highest was at 7.49% in T<sub>5</sub>. In conclusion, CP, EE, essential amino acid, and polyunsaturated (PUFA) fatty acid proportion increased with the advancement of dental age maturity and conversely, saturated fatty acid (SFA) proportion was decreased. Therefore, this research will play a vital role to characterize accurately in terms of its nutritional quality to be aware of the selection of indigenous beef in their daily diet according to dental age.

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### Introduction

A crucial component of agriculture, livestock contributes to the growth and improvement of Bangladesh's agricultural economy in a multitude of ways, involving the provision of food, revenue, savings, nourishment, social, cultural, manure, transport and draught power purposes (Hossain et al., 2016; Islam et al., 2022a; Kamal et al., 2022). The primary source of protein in developing nations like Bangladesh is meat (Haque et al., 2017; Sarker et al., 2021). The report states that each head requires 120 gm of meat per day (DLS, 2022) and beef share a larger proportion of this requirement. Beef is a crucial source of nutrients for the diet, (Zanovec et al., 2010; O'Neil et al., 2011; Rahman et al., 2020; Islam et al., 2022b) particularly high-quality protein, so eating beef may be healthful. All of the essential amino acids, as well as healthy minerals and vitamins including vitamin B12, are found in meat protein (Sadakuzzaman et al., 2021). By enhancing iron absorption and reducing calcium losses, animal protein plays a vital physiological role (Piskin et al., 2022). Although beef provides a special package, people are eating less of it (Davis and Lin 2005). This decline could have been impacted by how consumers see beef's place in a balanced diet (Guenther et al., 2005; Yen et al., 2008). Due to the high levels of saturated fatty acids and cholesterol in beef and other red meats, some consumers feel that they should be avoided (Guenther et al., 2005). Others may be worried about the usage of steroids or medications in beef cattle, as well as the increased risk of cancer (Tasevska et al., 2009). According to (International Food Information Council Foundation, 2009), recent statistics from a U.S. poll, 63% of consumers are interested in eating lower animal fat and between 2002 and 2008, 41% are thought to have consumed less beef (American Dietetic Association, 2008).

New findings from systematic research studies, however, revealed that there is inadequate evidence regarding the association of SFA to coronary heart disease (CHD) (Hunter et al., 2010; Micha and Mozaffarian, 2010; Siri-Tarino et al., 2010). The worries about compounds used in cattle production are responsible for cancer risk aren't backed up by reliable research (Koutros et al., 2008). According to the 2010 Dietary Guidelines Advisory Committee Report (USDA, 2010) and the American Heart Association's guidelines (Gidding et al., 2009), all healthy Americans should limit their SFA consumption to less than 10% of calories and their cholesterol intake to less than 300 mg per day. The statement is frequently interpreted by consumers as advising them that they should avoid eating red meats specially beef (Guenther et al., 2005). A report by the Institute of Medicine's Food and Nutrition Board (2002, 2005), only thirty percent of the fatty acids in beef enhance cholesterol levels, while stearic acid, which accounts for thirty percent of the SFA in beef, has no effect on serum cholesterol levels (Kris-Etherton et al., 2005; Daley et al., 2010). The single most abundant source of monounsaturated fatty acids (MUFA) in the diet is beef (Cotton et al., 2004). Due to its simplicity in a healthy diet and interchangeability with other lean red meats, lean beef may play a significant positive role in the diet (Wolmarans et al., 1999; Cassady et al., 2007).

In particular, during the fetal period, meat provides polyunsaturated fatty acids that provide from meat are play an important role in brain development (Sarker et al., 2021). Consumers are becoming increasingly aware of the truth as beef quality is not distinct and the fact that several elements need to be taken into account in order to improve the nutritional value of the diet of humans. The role of beef, particularly lean meat, in the diet has not been researched in depth.

Therefore, it is necessary that the nutritional value and safety of our indigenous beef be effectively identified according to dental age for eating. The ability to choose beef wisely for their daily diets depends on their perception of the origin, chemical makeup, and nutritional value of beef. The current study covers crucial topics pertaining to the nutritional value and gastronomic merits of our indigenous beef. The ante mortem (dental age) and post-mortem (ageing) influences on both the physical and biochemical attributes that determine beef quality are summarized. One of the primary characteristics of small-scale beef farming that delivers the formal or irregular marketplace is the absence of documents for finding out the years of age of cattle for sales. On the black market, there are unidentified-age animals for sale, and it has been very difficult to price them (Van Rooyen et al., 2007). The accepted standards for sheep and goats have typically been used to determine the age of beef cattle through their dentition (Wilson and Durkin, 1984). Permanent teeth replace deciduous (milk) teeth, which emerge first. The time of the eruption, or when teeth first break through the gums, is likely the most reliable indicator of an animal's age when no other reliable data are available. Knowing an animal's age is important since it has been shown to alter the amount of offal, edible body parts, and carcass yield in beef cattle (Aduku et al., 1991; Skapetas et al., 2006). The objective of this study was to know the effect of dental age on the nutritional quality such as proximate composition, amino acid and fatty acid profiles of beef of indigenous cattle.

## **Materials and Methods**

### **Carcass background**

An indigenous Bangladeshi bull with an unknown nutritional history was chosen for the slaughter line to investigate the physicochemical properties and nutritional condition of meat. Cattle with different dental ages were collected from different feedlots over the country. The slaughtering operation was conducted at the Mini Slaughter plant, at Department of Animal Science, Bangladesh Agricultural University, Mymensingh.

### **Carcass data collection**

Cattle were slaughtered weekly, the left halves of carcass were categorized, based on maturity and afterwards one side of each group was selected arbitrary. According to dental maturity, the carcasses were grouped into five categories and subjected to a completely randomized design (0, 2, 4, 6 or 8 permanent incisors). About 2 kg cranial extremity of the boneless ribeye was removed once the carcass had cooled, and then labelled. It was then taken to the lab for examination after being frozen at -18°C.

### **Nutritional analysis**

There are several techniques for evaluating the nutritional value and meat quality, each of which is based on a particular set of equipment, probes, and/or concepts. Prior to and throughout the analysis, special care was taken to standardize the handling and processing of meat samples. The results obtained in various research and laboratories were not always following the standard values, which was not surprising given the intricacy of meat processing during post-mortem time and quality trait determination. Therefore, precise adherence to the processing and measurement criteria is required for results comparability, which is why standardization is essential. A 12 cm thick, boneless longissimus part was excised from the tail end of the wholesale rib. Individually, vacuum-packed longissimus samples were frozen at -20°C. Two steak samples, measuring 2.54 cm thick, were created from each frozen longissimus sample (AMSA, 1995). After being pulverized in a ball mill, the proximate make up of each sample were examined.

### **Proximate composition**

For determination of moisture content, crude protein, total fat, and total ash, meat sample preparation was performed based on the method derived from AOAC, 1995. Duplicate samples were analyzed.

### **Amino acid determination**

During different growth stages, meat amino acid content was analyzed. AOAC official method 994.12 (first action 1994, final action 1997) was followed for the analysis. In order to determine dry matter content, the beef samples were dried in an oven for 24 h at 700°C and further 30 min at 105°C. The samples were then ground into a homogeneous size before being sieved with an opening of 0.25 mm and digested. Each digestion tube contained 10 mg nitrogen content (equivalent to 100 to 1000 mg of test portions). Twenty mL of Alpha-aminobutyric acid (AABA) solutions (2.5 mM) in 6 M HCl were combined with these samples and hydrolyzed at 115°C for 24 h. 500 µL of protein hydrolysate was cooled, then filtered through a 0.45 µm filter (Millipore Corporation, USA) into an eppendorf tube. Following nitrogen flash drying of ten µL of hydrolysate, a conventional technique was followed to derivatize it by ortho-phthaldialdehyde (OPA) reagent.

OPA and beta-mercaptoethanol were used in a pre-column derivatization method, followed by high-performance liquid chromatography (HPLC), to analyze the amino acids in the meat. The HPLC system is made by KNAUR in Germany and consists of two Model 64 micropumps, a Fluoromonitor III from TSP in the United States, a temperature controller module, and a degasser. The controller for the EUROCHROM 2000 chromatographic system handles data administration and acquisition. Eluent A, which is constituted of 93% 12.5 mM phosphate buffer and 7% acetonitrile, is replaced by Eluent B, which is 100% methanol, while the system runs under gradient conditions. A fluorescence detector is used for detection at Ex 340 nm and Em 455 nm. The system has a vortex column (Lichrospher 100 RP-18 encapped 5 µm) with an internal diameter of 250 x 4 mm and is maintained at a temperature of 40°C. Aspartic acid, glutamic acid, glycine, serine, threonine, methionine, valine, alanine, phenylalanine, isoleucine, leucine, arginine, lysine, and tyrosine are the 14 amino acids in meat samples that are efficiently resolved by the gradient condition for separation within a 55 min time frame (AOAC, 1997).

## Fatty acid analysis

We used the Robot Coupe S.N.C. R301 Ultra food processor, made in Vincennes, France, to defrost and homogenize the samples. Following Folch et al. (1957) procedure, muscle fat was separated from the homogenized muscle by adding butylated hydroxytoluene, which serves as an antioxidant, in a concentration of 0.05% (w/v) to a 2:1 (v/v) mixture of chloroform and methanol. Two gm of muscle were homogenized with 20 mL of solvent in an 80 mL screw-cap test tube (measured at 25 mm × 200 mm). An Ultra Turrax T25 homogenizer from IKA Labortechnik in Staufen, Germany, which was made by Janke and Kunkel, was used for this homogenization. A further 20 mL of the solvent solution were added after 16 mL of the solvent solution were used to rinse the homogenizer. The tube's contents were filtered using No. 4 Whatman filter paper from Whatman, Ltd. in Maidstone, United Kingdom. 5 mL of solvent solution was used to rinse the tubes, and a further 5 mL was used to rinse the filter cake. After that, 25% of the filtrate was added to the test tubes, which were then filled with a 0.02% solution of CaCl<sub>2</sub> in distilled water. These tubes were shaken before being let to separate for the entire night at a temperature of 4°C. After vacuum extraction of the upper aqueous layer, the lower layer was filtered through a funnel containing Whatman No. 4 filter paper and approximately 5 gm of anhydrous Na<sub>2</sub>SO<sub>4</sub>. The filtrate with the purified intramuscular lipid was extracted and kept overnight at 30°C in 50 mL glass screw-cap vials. The lipid substances were reconstituted in 1 mL of chloroform after being dehydrated to a uniform weight under a stream of N<sub>2</sub>. The solid-phase extraction cartridges (Bond-Elut 500 mg, 3 mL reservoir; Varian Instruments, Palo Alto, CA) were loaded with the lipid samples and packed with 500 mg of aminopropyl. A 3 mL by 3 mL chloroform flush had been used to condition these cartridges previously. The neutral lipid (NL) fraction was extracted using 4 mL of chloroform, and the eluate obtained was collected. The cartridges were cleaned with a 1:1 chloroform/methanol (v/v) mixture and 5 mL of methanol prior to the separation of the polar lipid (PL) fraction. When the NL and PL fractions were dehydrated to a uniform weight in pre-weighed glass tubes (12 mm × 75 mm), the weight of each fraction was recorded. The different lipid groups were dissolved in 300 µL of toluene to produce fatty acid methyl esters (FAME). Both alkaline and acidic transesterification were necessary for the methylation processes. As described by Park et al. in 2000, the recovered lipid fractions were first methylated with NaOCH<sub>3</sub> and then subjected to treatment with a 4% HCl solution in methanol. The purpose of this treatment was to avoid the probable isomerization of conjugated dienes that could result from the use of BF<sub>3</sub>/CH<sub>3</sub>OH. The two methylation processes were carried out for 20 min at a temperature of 50°C. For the purpose of measuring fatty acids, tricosanoic acid (C23:0) methyl ester was used as an internal reference. Deionized water was added to the tube containing the FAME in a ratio of 95:5 water to hexane (v/v), and then 2 mL of hexane. After being centrifuged at 800 g for five min, the top layer of the tubes that contained FAME in hexane was taken out and put into glass tubes (12 mm × 75 mm). This procedure was repeated with 2 mL of deionized water that had been steeped in hexane. The top layers were divided into tubes with about 0.75 gm of Na<sub>2</sub>SO<sub>4</sub> and centrifuged at 800 g for five min. Before injection, a 500 µL aliquot of the FAME-containing supernatant was placed into a 2 mL glass vial and further diluted with 500 µL of hexane.

## Gas chromatographic analysis

Using gas chromatography on a Varian 3800 GC outfitted with a CP-Sil 88 capillary column (100 m 0.25 mm i.d., 0.2 µm film thickness; Chrompack, The Netherlands) and a Varian 8400 autosampler, the FAME (Fatty Acid Methyl Esters) were separated. Temperatures of 250°C and 260°C, respectively, were consistently maintained for the injector and flame ionization detector. The column oven's temperature was first set at 40°C for 2 min, ramped up to 80°C and maintained there for 2 min, then raised to 160°C at 20°C/min, 220°C at 4°C/min, and finally 240°C at 2°C/min, where it was maintained for 8 min. The runtime was 43 min, with hydrogen gas serving as the carrier gas. For peak determination, a standard mixture consisting of 37 FAME (Fatty Acid Methyl Esters) from Supelco Inc. in Bellefonte, PA, was utilized. To identify FAME not included in the standard mixture, individual standards obtained from Matreya Inc. in Pleasant Gap, PA, were used.

## Statistical model and analysis

With five treatments and 10 replications of various animal age groups, the study was carried out utilizing a completely randomized methodology. A general linear model in a Completely Randomized Design (CRD) was used in an analysis of variance to determine the effect of dental age on the nutritional content of beef.

The SAS version 9.1 (SAS Institute, Inc.) GLM technique was used to analyze the data acquired from the experiment. Analysis of variance was used to determine the effect of dental maturity, and when significant differences were found, Duncan's Multiple Range Test (DMRT) was used to compare the treatment means with a significance threshold of 5%. In order to forecast carcass yield and find significant correlations between carcass features, Pearson's correlation coefficient was also performed (SAS, 1996).

## Results and Discussion

### Proximate composition of longissimus dorsi muscle

From the Table 1, it has been shown that the highest CP% was 84.91 having the T<sub>5</sub> while the lowest figure was 72.94 having T<sub>4</sub>. There was a significant effect of age on crude protein content which is aligned with a study by Hosain et al. (2015). In case of ash content, the highest amount was 4.41% with dental maturity of T<sub>1</sub> (0 pi) followed by 4.12%, 3.44%, 3.41%, and 3.27% with a dental maturity of T<sub>4</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>5</sub>, respectively. Ash content were impacted (p<0.01) by dental maturity. Similarly, (Table 1), on the concentration of ether extract (EE) of beef samples, age had a significant (p<0.001) impact. Beef from animals with T<sub>1</sub> had lower (3.34%) EE content in comparison to animals with teeth from the T<sub>2</sub> (3.37%), T<sub>4</sub> (3.43%), and T<sub>6</sub> (3.41%) teeth groups. The mean ether extract levels in maturity category 8 carcasses were 4.15%, which was different from the other groups (p<0.001; Table 1). Although meat from cattle had lower moisture means with T<sub>3</sub> and greater moisture means was 73.98% with T<sub>1</sub>, maturity had no effect on moisture content (p>0.05). The EE content of our indigenous beef was consistent with the conclusion made by Duarte et al. (2011) (Table 1). They explained their result that these animals had eight permanent incisors, and meat from them contained a greater amount of ether extract. Longissimus muscle's mean ether extract level fell within the recommended range suggested by Duarte et al. (2011) for tender, tasty, and juicy meat (more than 4%).

**Table 1.** Proximate composition of longissimus dorsi muscle of indigenous cattle (n=17)

Parameter (On DM basis)	T <sub>1</sub> Mean±SE	T <sub>2</sub> Mean±SE	T <sub>3</sub> Mean±SE	T <sub>4</sub> Mean±SE	T <sub>5</sub> Mean±SE	P value	Sig. level
Moisture%	73.98±0.61	71.89±0.78	71.53±0.33	71.93±0.75	72.74±0.05	0.0687	NS
DM%	26.04 <sup>b</sup> ±0.62	28.11 <sup>a</sup> ±0.78	28.47 <sup>a</sup> ±0.33	28.07 <sup>a</sup> ±0.75	27.27 <sup>ab</sup> ±0.05	0.072	*
CP%	83.17 <sup>ab</sup> ±1.90	75.22 <sup>bc</sup> ±3.69	77.12 <sup>abc</sup> ±3.53	72.94 <sup>c</sup> ±1.63	84.91 <sup>a</sup> ±1.05	0.0136	**
EE%	3.34 <sup>b</sup> ±0.07	3.37 <sup>b</sup> ±0.03	3.43 <sup>b</sup> ±0.02	3.41 <sup>b</sup> ±0.03	4.15 <sup>a</sup> ±0.13	<.0001	***
Total ash%	4.41 <sup>a</sup> ±0.29	3.41 <sup>b</sup> ±0.21	3.44 <sup>b</sup> ±0.12	4.12 <sup>a</sup> ±0.14	3.27 <sup>b</sup> ±0.07	0.0004	**
Ca%	0.02 <sup>ab</sup> ±0.00	0.02 <sup>b</sup> ±0.001	0.02 <sup>b</sup> ±0.00	0.03 <sup>a</sup> ±0.001	0.02 <sup>ab</sup> ±0.002	0.0478	*
P%	0.17±0.00	0.16±0.002	0.16±0.002	0.16±0.003	0.16±0.002	0.1504	NS
Carbohydrates% on fresh basis	2.39 <sup>bc</sup> ±0.56	5.14 <sup>a</sup> ±1.10	4.60 <sup>ab</sup> ±1.04	5.53 <sup>a</sup> ±0.56	2.09 <sup>c</sup> ±0.30	0.0104	**

T1 = 0 Permanent incisor, T2 = 2 Permanent incisors, T3 = 4 Permanent incisors, T4 = 6 Permanent incisors, T5 = 8 Permanent incisors; Means with different superscripts in a row differ significantly; NS= Non significant, \*\*\*=p<0.001, \*\*=p<0.01; \*=p <0.05.

### Amino acid profile

Only 12 amino acids could be analyzed using the approach in this investigation. Overall means and standard error of amino acids those of categorized by dental maturity category for longissimus dorsi muscle in between the samples of the 12<sup>th</sup> and 13<sup>th</sup> ribs are given in Table 2. The amino acid contents (g/100g of meat) of glutamic acid (16.58), serine (3.40), glycine (4.06), methionine (2.14), arginine (5.68), alanine (4.43), tyrosine (2.26), valine (7.72), phenylalanine (3.84), isoleucine (3.40), leucine (6.85) and lysine (8.16) with having the dental maturity of (0 pi) while the amino acid contents of glutamic acid (16.51), serine (3.33), glycine (4.18), methionine (2.56), arginine (6.56), alanine (5.23), tyrosine (2.77), valine (6.84), phenylalanine (3.61), isoleucine (4.46), leucine (8.72) and lysine (8.76) with having the dental maturity (8pi). Since acid hydrolysis destroys tryptophan, it was not assessed in this study. Regardless of age groups, it can be shown from the Table 2, glutamic acid was the main amino acids, followed by lysine, leucine, and arginine. Dental maturity of indigenous beef cattle had a significant effect at the 5% level on the content of serine, arginine, methionine, lysine, isoleucine, leucine and alanine amino acids of longissimus dorsi muscle. On the other hand, dental maturity had no significant effect on the contents of glutamic, glycine, tyrosine, valine, and phenyl alkaline acids of the longissimus dorsi muscle. Whereas, leucine, alanine, and valine content soared with advancing age, according to Schonfeldt et al. (2010). It also has been stated in Table 2 that the content of essential amino acids was increased with having 41.51 in T<sub>5</sub> (8 pi), followed by 40.35 (T<sub>4</sub>), 40.98 (T<sub>3</sub>), 39.03 (T<sub>2</sub>), and 37.79 (T<sub>1</sub>).

**Table 2.** Influence of dental age on amino acid profile of longissimus dorsi muscle of indigenous cattle (n=17)

Amino acid	T <sub>1</sub> Mean±SE	T <sub>2</sub> Mean±SE	T <sub>3</sub> Mean±SE	T <sub>4</sub> Mean±SE	T <sub>5</sub> Mean±SE	P value	Sig. level
Glu	16.58±0.82	14.12±1.23	15.43±0.33	15.11±0.14	16.51±0.31	0.1249	NS
Ser	3.41 <sup>a</sup> ±0.32	2.54 <sup>b</sup> ±0.11	2.93 <sup>ab</sup> ±0.25	2.72 <sup>ab</sup> ±0.08	3.33 <sup>a</sup> ±0.01	0.0458	*
Gly	4.06±0.04	3.85±0.06	4.05±0.08	3.87±0.13	4.18±0.01	0.0767	NS
Met	2.14 <sup>c</sup> ±0.06	2.29 <sup>bc</sup> ±0.08	2.39 <sup>ab</sup> ±0.05	2.46 <sup>ab</sup> ±0.09	2.56 <sup>a</sup> ±0.01	0.0075	**
Arg	5.68 <sup>b</sup> ±0.30	5.77 <sup>b</sup> ±0.22	6.63 <sup>a</sup> ±0.02	6.62 <sup>a</sup> ±0.17	6.56 <sup>a</sup> ±0.01	0.0083	**
Ala	4.43 <sup>b</sup> ±0.23	3.55 <sup>c</sup> ±0.11	4.62 <sup>ab</sup> ±0.32	3.75 <sup>c</sup> ±0.18	5.23 <sup>a</sup> ±0.00	0.0008	**
Tyr	2.26±0.03	2.30±0.03	2.71±0.35	2.39±0.03	2.77±0.13	0.0951	NS
Val	7.72±0.31	7.36±0.43	7.08±0.26	7.33±0.28	6.84±0.05	0.3548	NS
Phe	3.84±0.21	3.44±0.03	3.68±0.05	3.71±0.03	3.61±0.10	0.2885	NS
Ile	3.40 <sup>b</sup> ±0.11	3.37 <sup>b</sup> ±0.13	4.15 <sup>a</sup> ±0.18	3.22 <sup>b</sup> ±0.01	4.46 <sup>a</sup> ±0.06	<.0001	***
Leu	6.85 <sup>b</sup> ±0.33	8.22 <sup>a</sup> ±0.13	8.48 <sup>a</sup> ±0.29	8.31 <sup>a</sup> ±0.22	8.72 <sup>a</sup> ±0.08	0.001	**
Lys	8.16 <sup>b</sup> ±0.07	8.58 <sup>a</sup> ±0.20	8.57 <sup>a</sup> ±0.01	8.70 <sup>a</sup> ±0.01	8.76 <sup>a</sup> ±0.07	0.0027	**
Total	68.52 <sup>b</sup> ±0.60	65.39 <sup>c</sup> ±0.38	70.73 <sup>b</sup> ±1.52	68.18 <sup>b</sup> ±0.73	73.53 <sup>a</sup> ±0.37	0.0003	**
Essential	37.79 <sup>c</sup> ±0.68	39.03 <sup>bc</sup> ±0.88	40.98 <sup>a</sup> ±0.23	40.35 <sup>ab</sup> ±0.35	41.51 <sup>a</sup> ±0.20	0.0024	**
Non-essential	30.74 <sup>ab</sup> ±1.01	26.37 <sup>c</sup> ±1.26	29.74 <sup>ab</sup> ±1.29	27.83 <sup>bc</sup> ±0.37	32.02 <sup>a</sup> ±0.32	0.008	**

T1 = 0 Permanent incisor, T2 = 2 Permanent incisors, T3 = 4 Permanent incisors, T4 = 6 Permanent incisors, T5 = 8 Permanent incisors; Means with different superscripts in a row differ significantly; NS= Non significant, \*\*\*=p<0.001, \*\*=p<0.01; \*=p <0.05; Glu=Glutamic acid, Ser= Serine, Gly= Glycine, Met= Methionine, Arg= Arginine, Ala= Alanine, Tyr= Tyrosine, Val= Valine, Phe= Phenylalanine, Ile= Isoleucine, Leu= Leucine and Lys= Lysine; Essential amino acid= Sum of Met, Arg, Val, Phe, Ile, Leu and Lys; Non-essential amino acid= Sum of Glu, Ser, Gly, Ala and Tyr.

The above-stated essential amino acid contents show a clear increasing trend with the advances of dental maturity except for the dental maturity group of 6pi and it has a significant effect at 1% level on essential amino acid. This partially similar trend was also followed in case of non-essential amino acid where the content of non-essential amino acid was increased having 32.02 with T<sub>5</sub> (8 pi), followed by 27.83 (T<sub>4</sub>), 29.74 (T<sub>3</sub>), 26.37 (T<sub>2</sub>) and 30.74 (T<sub>1</sub>). The above stated essential amino acid contents show a clear increasing trend with the advances of dental maturity except the dental maturity group of 0pi (30.74) and it has a significant effect at 1% level on the non-essential amino acids. It was established in the study of Zakhariet et al. (1980) that using a Czechoslovakia-made automatic amino analyzer Hd 1200 E, it was possible to identify the amino acid compositions (both quantitative and qualitative) in the proteins of muscle (longissimus dorsi) from cattle of the "Bulgarian Brown" breed. A slight difference is found between the amount of amino acid content of the results of the current investigation and those of earlier studies conducted by Zakhariet et al. (1980). Samicho et al. (2013) studied in their experiment that the flank cut of the beef component had considerably greater levels of serine, aspartic acid, arginine, glutamic acid, threonine, leucine, lysine, isoleucine, methionine, valine, and phenylalanine which is somewhat compatible with our findings. In our study, the dental maturity of indigenous beef cattle had a significant effect at the 5% level on the content of serine, arginine, methionine, lysine, isoleucine,



leucine and alanine acids of muscle (longissimus dorsi) presented in Table 2. In addition, this conclusion was partially supported by a study by Greenwood et al. (1951) on the amino acid composition of raw and cooked beef slices. The aforementioned researchers backed the current research findings since we were limited in our capacity to identify more amino acids due to instrumentation. Various cow species, animal ages, the effects of gender (Hollo et al., 2001), feeding high or low amino acid compounds, slaughter weight, and processing (Lawrie, 1998), may further contribute to this explanation. In conclusion, there exists no consistent relationship between the concentration of amino acids in beef and dental maturity. In decreasing order, glutamic acid, valine, lysine, leucine, arginine, glycine, and phenylalanine were the most prevalent amino acids in beef. Present findings are fully consistent with the study done by Samicho et al. (2013). However, methionine was present in much lower amounts which was in agreement with (Greenwood et al., 1951). The essential amino acid comprised more than 40% of the total amino acid content of the longissimus dorsi muscle which was in agreement with (Samicho et al., 2013).

### Fatty acid profile

Table 3 shows the proportion of fatty acids in LM intramuscular fat having the different age groups of indigenous beef cattle. In the LM of beef, the majority of fatty acids were in the form of palmitic acid (16:0), stearic acid (18:0), and oleic acid (18:1) irrespective of all age groups having T<sub>1</sub> (0 pi) to T<sub>5</sub>(8 pi). Oleic acid, which is prevalent in intramuscular fat found by (Hwang and Joo, 2017). Linoleic (C18:2 n-6) acid proportion of beef LM was greater in the T<sub>5</sub> (6.70%) than that of T<sub>1</sub> (0.72%) (P<0.05). Fatty acids nature and quantity in beef flesh greatly influence how well-done it tastes and how soft and flavorful it is (Frank et al., 2016). The Linoleic acid (C18:2 n-6) proportion in other dental age groups was observed in the T<sub>2</sub> (1.74%), T<sub>3</sub> (3.08%), and T<sub>4</sub> (2.47%). The greatest proportion of beef LM linolenic fatty acid (C18:3 n-3) was observed in the T<sub>3</sub> (0.74%) and the lowest was in T<sub>4</sub> (0.37%) (P < 0.05). The linolenic acid (C18:3 n-3) proportion in other dental age groups was observed in T<sub>1</sub> (0.62%), T<sub>2</sub> (0.49%), and T<sub>5</sub> (0.58%). Beef LM having T<sub>2</sub> had also the greatest proportion of eicosapentaenoic (C20:5 n-3, 0.41%) than that of T<sub>1</sub> (0.18%). The eicosapentaenoic (C20:5 n-3) acid proportion in other dental age groups was observed in T<sub>3</sub> (0.35%), T<sub>4</sub> (0.37%), and T<sub>5</sub> (0.22%). Dental maturity has no significant effect on eicosapentaenoic (C20:5 n-3) acid. Linolenic (C18:3 n-3) and Linoleic (C18:2 n-6) fatty acid proportions of beef LM differed significantly (p<0.05) among all dental age maturity groups. In the Table 3, the total polyunsaturated fatty acid (PUFA) proportion of beef LM was lowest in T<sub>1</sub> (1.52%) and highest (p<0.05) in T<sub>5</sub> (7.49%). The total polyunsaturated fatty acid (PUFA) proportion of other dental age groups was observed in T<sub>2</sub> (2.64%), T<sub>3</sub> (4.17%), and T<sub>4</sub> (3.21%). The total polyunsaturated fatty acid (PUFA) proportion of beef LM differed significantly (p<0.05) among all dental age maturity groups. Total n-3 and n-6 PUFA proportions were higher in T<sub>5</sub> than the all other dental maturity age groups with exception of T<sub>1</sub> for total n-6 PUFA proportions. Scheeder et al. (2001) found 23.55% (16:0), 21.08% (18:0), and 38.77% (18:1 c-9) fatty acid proportions in confined Brown Swiss bulls. It also supports our findings in the Table 3. It has also been stated in Table 3 that the total polyunsaturated fatty acid (PUFA) proportion of beef LM was lowest in T<sub>1</sub> (1.52%) and highest (p<0.05) in T<sub>5</sub> (7.49%). The total polyunsaturated fatty acid (PUFA) proportion of beef LM differed significantly (p<0.05) among all dental age maturity groups. Pavan et al. (2013) found 2.34% and 1.21% of the PUFAs group of LM, 18:2 n-6 and 18:3 n-3, respectively, in retail slices of grass-fed systems for growing beef. This has been consistent with our results having the dental age maturity group of T<sub>4</sub>, followed by a little bit lower in T<sub>1</sub> and T<sub>2</sub>; and higher in T<sub>3</sub> and T<sub>5</sub> for the case of 18:2 n-6. But in the case of 18:3 n-3, it is a well in agreement with the results of Roseli et al. (2006) who documented that in the LM of bull and steer, it was observed 0.85% and 0.53% 18:3 n-3, respectively. Roseli et al. (2006) also stated that in the PUFAs group, 18:3 n-3 and 18:2 n-6 predominated and bulls showed higher 18:3 n-3 and 18:2 n-6 content in comparison to steers. Roseli et al. (2006) also stated that total PUFA content in LM of the steers and bulls studied was similar which is not consistent with our findings. In our studies, in Table 3 it has been shown that the total polyunsaturated fatty acid (PUFA) proportion of beef LM differed significantly (p<0.05) among all dental age maturity groups.

**Table 3.** Effect of dental age on fatty acid profile of longissimus dorsi muscle of indigenous cattle for beef (n=17)

Fatty acid	T <sub>1</sub> Mean±SE	T <sub>2</sub> Mean±SE	T <sub>3</sub> Mean±SE	T <sub>4</sub> Mean±SE	T <sub>5</sub> Mean±SE	P value	Sig. level
C12:0	0.07±0.02	0.07±0.01	0.09±0.00	0.07±0.00	0.06±0.01	0.6981	NS
C14:0	2.67±0.63	2.57±0.13	2.79±0.53	3.09±0.35	2.80±0.47	0.9451	NS
C15:0	0.71±0.14	0.35±0.13	0.27±0.09	0.42±0.07	0.47±0.21	0.2566	NS
C16:0	23.78±2.98	25.23±1.79	25.10±1.67	26.92±1.17	24.93±2.09	0.8198	NS
C17:0	1.22±0.13	1.09±0.71	0.50±0.09	1.03±0.37	1.16±0.01	0.5103	NS
C18:0	23.08±3.59	16.25±2.55	18.17±1.13	17.55±3.07	16.51±0.11	0.4288	NS
C20:0	0.37 <sup>b</sup> ±0.06	0.80 <sup>a</sup> ±0.09	0.25 <sup>b</sup> ±0.03	0.28 <sup>b</sup> ±0.03	0.31 <sup>b</sup> ±0.02	0.0001	***
SFA	51.89±4.34	46.37±5.39	47.17±2.65	49.37±2.66	46.25±2.42	0.7358	NS
C14:1	0.47±0.11	0.55±0.10	0.67±0.01	0.57±0.01	0.44±0.03	0.1048	NS
C15:1	0.23±0.06	0.26±0.01	0.24±0.04	0.23±0.01	0.24±0.00	0.9613	NS
C16:1	4.31±1.61	5.42±0.03	4.38±0.15	4.17±0.91	4.80±1.09	0.9366	NS
C17:1	1.04±0.29	1.09±0.11	1.45±0.60	0.97±0.50	1.06±0.20	0.9378	NS
C18:1	46.36±4.76	59.36±5.43	45.51±1.04	47.18±2.70	45.15±1.65	0.1043	NS
C20:1	0.22±0.03	0.20±0.02	0.31±0.02	0.28±0.02	0.39±0.19	0.6387	NS
MUFA	52.64±6.11	66.87±5.45	52.57±1.59	53.38±3.61	52.07±1.45	0.192	NS
C18:2	0.72 <sup>b</sup> ±0.15	1.74 <sup>b</sup> ±1.25	3.08 <sup>ab</sup> ±1.42	2.47 <sup>b</sup> ±0.59	6.7 <sup>a</sup> ±2.014	0.0499	*
C18:3	0.62 <sup>ab</sup> ±0.04	0.49 <sup>ab</sup> ±0.06	0.74 <sup>a</sup> ±0.04	0.37 <sup>b</sup> ±0.02	0.58 <sup>ab</sup> ±0.15	0.0345	*
C20:5	0.18±0.02	0.41±0.18	0.35±0.05	0.37±0.04	0.22±0.07	0.1503	NS
PUFA	1.52 <sup>b</sup> ±0.13	2.64 <sup>b</sup> ±1.37	4.17 <sup>ab</sup> ±1.33	3.21 <sup>b</sup> ±0.60	7.49 <sup>a</sup> ±1.94	0.043	*
UFA	54.15±6.23	69.51±6.82	56.74±2.88	56.59±3.39	59.56±3.35	0.3012	NS

T<sub>1</sub> = 0 Permanent incisor, T<sub>2</sub> = 2 Permanent incisors, T<sub>3</sub> = 4 Permanent incisors, T<sub>4</sub> = 6 Permanent incisors, T<sub>5</sub> = 8 Permanent incisors; Means with different superscripts in a row differ significantly; NS= Non significant, \*\*=p<0.01; \*p <0.05; C14:0= Myristic acid, 16:0= palmitic acid, 18:0= stearic acid, 18:1 = oleic acid, C18:2 = Linoleic acid, C18:3= Linolenic fatty acid, C20:5 = Eicosapentaenoic acid, SFA= Saturated fatty acid and sum of C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, MUFA= Monounsaturated fatty acid and sum of C14:1, C15:1, C16:1, C17:1, C18:1 and C20:1, PUFA= Polyunsaturated fatty acid and sum of C18:2, C18:3 and C20:5, UFA= Unsaturated fatty acid and sum of MUFA and PUFA.

## Conclusions

It may be concluded that the amount of CP, EE, essential amino acids, and polyunsaturated (PUFA) fatty acids increased with the advancement of dental age, whereas the proportion of saturated fatty acids (SFA) declined. In addition, the nutritional quality of cattle with 8 permanent incisors was superior compared to other dental groups.

## Conflicts of Interest

The authors declare no potential conflict of interest.

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