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REGULAR ARTICLES

Fatty acid and conjugated linoleic acid content of Anatolian buffaloes at different muscle types and slaughter weight

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Abstract

This research was performed to detect tissue fatty acid (FA) composition and conjugated linoleic acid (CLA) content in Anatolian buffaloes at different muscle types (MTs) and slaughter weights (SWs). The research was carried out on a private commercial livestock farm in Tokat. The research's animal material comprised 20 Anatolian buffalo calves with approximately 100 kg body weights, weaned at about 150 days of age. Before the experiment started, the calves were randomly divided into two slaughter groups (SW) as low weight (LW=225 kg) and high weight (HW=325 kg). Ten calves from each of the two experimental groups, which were intensively fed, were slaughtered in two different weights. After the slaughtering, FA composition and CLA content of the *Semimembranosus (SM), Semitendinosus (ST)*, and *Triceps brachii (TB)* muscle tissues of the animals were examined. The study determined that palmitoleic acid (C16:1) and stearic acid (C18:0) were affected by MTs and oleic acid (C18:1) and α-linolenic acid (C18:3 n-3) were affected by SW ($P<0.05$). The highest CLA was in the ST muscle type (0.298) and the LW group (0.289) of the SW groups ($P>0.05$). With the increase of SW (in LW and HW groups), n-6/n3 (SM: 7.783 and 6.533; ST: 8.115 and 7.859; TB: 8.416 and 8.215) ($P>0.05$) and PUFA ratio decreased ($P<0.05$). The SW increase raised the SFA ratio in the SM muscle ($P<0.05$) while lowering it in the TB muscle ($P>0.05$). Again, with the increase in SW, AI and TI values increased in SM and ST muscles, while the same index values decreased in TB muscle ($P>0.05$). In conclusion, when considering the PUFA/SFA ratio and the beneficial effects of CLAs on human health, ST in the MT and LW groups in SW, and thus ST and LW in MT and SW were prominent in Anatolian buffaloes.

Keywords Anatolian buffalo calves · Fatty acids · Muscle type · Conjugated linoleic acid

Introduction

Nowadays, while scientists state the benefits of foods of animal origin, especially animal-origin fats, in human nutrition also express the risks and concerns (Taşçı 2019). Fats in the structures of cells, tissues, and organs in the human body are crucial nutrients to sustain life and perform various functions of the body healthily. The quality of this nutrient may affect consumers' animal food preferences, depending on the fatty acid (FA) content, which is one of the main structural components of fats (Mol 2008). FAs have positive and negative effects on human health due to their oxidative

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stability, cis/trans structure, saturated or unsaturated structure, and essential FA content. Some FAs have been reported to reduce blood cholesterol levels, while some FAs increase it. Especially omega 6 (n-6) and omega 3 (n-3) FAs, which are essential FAs, have been documented to protect cardiovascular health by reducing the amount of low-density lipoprotein (LDL) and triglycerides in the blood (Yakan and Ünal 2010; Aksoy et al. 2021). Therefore, the Department of Health and many researchers have recommended that PUFA/SFA) content should be 0.45 or higher, atherogenic (AI) and thrombogenic index (TI) values, as low as possible (less than 1), and the n-6:n-3 ratio, 4-5:1 or 1:1 in healthy diets (Ulbricht and Southgate 1991; Anonymous 1994a; Simopoulos 2002; Lazzaroni et al. 2009; Yakan and Ünal 2010; Aksoy et al. 2021).

CLA is one of the functional nutrients with positive effects on human health, and it is the positional and structural isomer of linoleic acid (C18:2, cis-9, cis-12) which is a natural component of foods obtained from ruminant animals and is included in PUFAs (Çelebi and Kaya 2008; Lehnen et al. 2015). Many researchers have reported that CLA prevents cancer, cardiovascular, atherosclerosis, and diabetic diseases, improves hyperinsulinemia, increases the immune system and bone density, and reduces obesity (Mcguire and Mcguire 2000; Whigham et al. 2000; Banni 2002; Khanal 2004; Köknaroğlu 2007; Çelebi and Kaya 2008; Dilzer and Park 2012; Cutrignelli et al. 2013; Alabiso et al. 2020). CLA, which are PUFA isomers, is found in milk and dairy products and red meat of ruminant animals such as cattle, buffalo, sheep, and goats (Gonçalves et al. 2010; den Hartigh 2019; Basak and Duttaroy 2020). Although the highest amount of CLA among meats is in lamb (8.8–19 mg g^{-1} FAME), the amount of CLA in other red meats has been reported to vary between 2.7 and 6.5 mg g^{-1} FAME (Schmid et al. 2006). The CLA content was determined to change between 2.0 and 12.7 mg g^{-1} FAME in different breeds and various MTs in buffaloes (Giuffrida de Mendoza et al. 2005; Cutrignelli et al. 2013; Cifuni et al. 2014; Calabrò et al. 2014; Di Stasio and Brugiapaglia 2021). Red meat is one of the healthiest and most crucial foods in the population's nutrition of all ages. It is rich in exogenous amino acids, easy to produce, pleases in taste, delicious, satisfies hunger quickly, contains sufficient amounts of vital nutrients in its structure, and thus quickly prevents nutritional disorders and diseases (Scollan et al. 2006; Pereira and Vicente 2013; Ye et al. 2020). One of the red meat and milk production sources in Türkiye is buffaloes. The buffaloes breed in Türkiye is called Anatolian buffaloes, whose origins date back to the Mediterranean buffaloes (MB), a sub-branch of the river buffaloes (Soysal 2009). As reported, the average carcass weight of Anatolian buffaloes is 215 kg (Ulutaş et al. 2021). Like carcass composition, the condition of the meat and fat in the carcass indicates the carcass quality (Grossi et al. 2013; Lambertz et al. 2014). Buffalo carcass has a different fat distribution than a beef carcass. Fat is scarce in the subcutaneous region in buffaloes (Tamburrano et al. 2019). It has been declared that buffalo meat contains more monounsaturated fatty acids (MUFAs) and PUFAs than beef (Sinclair et al. 1982; Cutrignelli et al. 2013). In some studies conducted in recent years, buffalo meat has gained increasing popularity due to its conspicuous beneficial properties. It has been defined as the healthiest meat for human consumption among red meats because it contains less fat and cholesterol than other red meats (Kandeepan et al. 2013; Di Stasio and Brugiapaglia 2021). The fatty acid content of meat is closely related to human health. Therefore, knowing the FA composition of meat consumed as human food in detail is extremely important in consumer preferences (Simopoulos 2016; Basak and Duttaroy 2020). In recent years, the quality of animal products (such as milk, meat) has gained importance as much as their quantity. Buffalo meat contains less SFA and intramuscular fat than beef (Valin et al. 1984; Cutrignelli et al. 2013; Tamburrano et al. 2019). Determining the FA content of Anatolian buffalo meat, an alternative red meat production source, will increase awareness among consumers about buffalo meat, and thus, this meat will take its rightful place in consumers' preferences. So far, there has been no detailed comparative literature research on different tissue FAs and CLA content of Anatolian buffaloes, whose number is increasing day by day through breeding programs in Türkiye. Many factors, such as slaughter weight (SW) (Holló et al. 2001; Yakan and Ünal 2010; Aksoy and Ulutaş 2016; Nogalski et al. 2014; Kul et al. 2020; Mottin et al. 2020; Aksoy et al. 2021) and anatomical region (Talpur et al. 2007; Karabacak et al. 2012; Calabrò et al. 2014; Kiani and Fallah 2016; Aksoy et al. 2019; Razmaite et al. 2019), affect tissue FA composition in livestock. Our previous studies had concluded that slaughter weight (SW) significantly affects the carcass (Ulutaş et al. 2021) and meat quality characteristics (Aksoy et al. 2021) of male Anatolian buffaloes. So far, a limited number of studies have investigated Anatolian buffaloe carcass (Ulutaş et al. 2021; Turan et al. 2021) and meat quality characteristics (Ekiz et al. 2018; Gecgel et al. 2019; Aksoy et al. 2021; Turan et al. 2021). However, no study examining Anatolian buffaloe tissue fatty acid compositions has been found in the literature. Previous works have investigated the fatty acid composition of *m. longissimus dorsi* tissue (Gecgel et al. 2019; Aksoy et al. 2021). In addition, very few studies have compared the CLA and FA contents of buffalo meats according to different SWs and muscle types (MT) (Cutrignelli et al. 2013; Cifuni

et al. 2014; Calabrò et al. 2014). Therefore, the current study investigated the tissue FA profile and CLAs content in Anatolian buffaloes at different SWs and MTs.

Materials and methods

Ethical approval

Kırşehir Ahi Evran University Local Animal Ethics Committee reviewed and approved the study's protocol on February 12, 2014 (number of decision 7 (1–4)). This protocol was taken into account in all the research processes.

Research area

The research was carried out in a private commercial livestock farm in the Turhal district of Tokat City, at 40°30′47″ north latitudes and 36°32′01″ east longitudes and 493 m above sea level. The average annual temperature in Tokat is 12.8 °C. While the highest temperature is 45 °C, the lowest is − 23.4 °C. The average yearly precipitation is 36.27 mm, and the recorded maximum is 59.1 mm in May.

Animal material

The research material comprised 20 Anatolian male buffalo calves, which were weaned at approximately 150 days of age and 100 kg body weights. In the study, the calves were divided into two groups as low and high. Anatolian male buffalo calves, whose SW had been determined beforehand, were randomly distributed to slaughter groups of ten buffaloes each group. Target slaughter weights of the buffaloes were 225 kg for the LW group and 325 kg for the HW group. During the study, the calves in both SW groups were fed until they reached the target weights.

Calf feeding and management

Before starting the experiment, calves were medicated against internal parasites (two per 100 kg calf [oxfendazole (250 mg) + oxyclozanide (750 mg)] and against external parasites [(0.2 mg kg^{-1} body weight (10 mg ml^{-1} ivermectin)]. Before the study, calves had a 7-day adaptation feeding. In the study, calves were intensively fattened with cattle feed (16.40% HP and 11,287.6 kJ kg^{-1} ME) and dried alfalfa grass (18.07% HP and 9152.3 kJ kg^{-1} ME) until they reached the target SW.

During the feeding period, the calves were fed ad libitum with mixed-feed containing 16.40% crude protein and alfalfa straw with 18.07% protein. One kilogram of the ration included 700 g of mixed feed and 300 g of dry alfalfa straw prepared according to NRC (1996). The calves had limitless water and licking stones in the fattening.

Determination of slaughter weight

The slaughtering was performed in a licensed commercial slaughterhouse by the standard procedure in Türkiye (TSI 1987). Slaughter weights were determined after weighing the calves in 2-week periods (12-h unfed). Calves were housed in 20 m^2 paddocks in the commercial livestock farm. When they were at their predetermined slaughter weight, they were transferred to the slaughterhouse 10 km away from the farm. Ten calves at the target weights were slaughtered out of each group.

Obtaining muscle samples

After the slaughter, the carcasses were kept at + 4 °C for 24 h. Later, the SM, ST, and TB muscle samples were taken from the left half of the carcass and isolated. Then, 150 g of samples were obtained from each muscle, and their fatty and connective tissues were removed. These muscle samples were kept in a deep freezer at ⁻20 °C till the analysis day.

Determination of the fatty and conjugated linoleic acids content

The lipid extraction from SM, ST, and TB muscles was carried out according to the method of Folch et al. (1957) (chloroform/methanol (2/1; v/v)). FA methyl esters (FAME) analysis was performed according to the International Union of Pure and Applied Chemistry (IUPAC) (Pacquot and Hautfenne 1990). A gas chromatography (GC) device determined FAME in all three muscles. A silica capillary column was utilized in the GC device (DB-23, 60 m long, 0.25 mm inner diameter (ID), and 0.25 µm film thickness). The column, injector, and detector temperatures were set at 190 °C, 230 °C, and 240° C, respectively. The gas split ratio was established as 60:1 for FAME determination, and the carrier gas was helium (0.5 ml min^{-1}). Supelco 37 FAME mixture (C4-C24) was utilized as a model to define FAMEs peaks. The results were g 100 g^{-1} (wt (%)) in FAME. In the same muscle group, 9c-11t and 10t-12c CLA isomers were determined using an external standard (Sigma Aldrich).

Determination of quality indices

In the study, for two SW and three MTs, AI (Formula 1), TI (Formula 2) (Ulbritcht and Southgate 1991; Cutrignelli et al. 2013; Calabrò et al. 2014; Chen and Liu 2020), desirable fatty acids (DFA) (Formula 3), and nutritive value (NV)

(Formula 4) were calculated according to Yakan and Ünal (2010).

$$AI = \frac{C12:0(4 \times C14:0) + C16:0}{MUFA + n-3PUFA + n-6PUFA} \quad (1)$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{\left(\frac{MUFA}{2}\right) + \left(\frac{n-6PUFA}{2}\right) + (3 \times n-3PUFA) + \left(\frac{n-3PUFA}{n-6PUFA}\right)} \quad (2)$$

$$DFA = C18:0 + MUFA + PUFA \quad (3)$$

$$NV = \frac{C18:0 + C18:1}{C16:0} \quad (4)$$

Evaluation of all data

The study data were evaluated by factorial experiment using the mixed model method. For this purpose, the PROC MIXED procedure of the SAS statistics program was utilized (SAS, 1999). The DUNCAN test was utilized to compare the means of muscle types and the *t*-test to compare the averages of SW groups. All analyses are made using the following formula.

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk}$$

In the formula, Y_{ijk}, each of the examined variables (CLAs and FAs), μ: least-squares mean, fixed effect according to the SW groups (*i*: fixed effect according to the SW group (*i*: 1st SW 225 kg live weight, *i*: 2nd SW 325 kg live weight), b_j: fixed effect according to MT (SM, ST and TB), $(ab)_{ij}$: interaction between SW and MT, e_{ijk}: random error.

The *t*-test was used to compare the subgroup averages related to SW, and the DUNCAN test was used to compare the subgroup averages regarding MT.

Results

The study shows the tissue FA composition of Anatolian buffaloes according to MTs (SM, ST, and TB) and SWs (LW and HW) in Tables 1 and 2. The variation of SFA (x), PUFA/SFA (y), and MUFA/SFA (z) contents according to different muscle types at different slaughter weights is given in Fig. 1. The variation of PUFA/SFA and n-6/n-3 ratios according to different muscle types at different slaughter weights is given in Fig. 2. The study found that MTs had an effect on palmitoleic acid (C16:1) and stearic acid (C18:0), while SW on oleic acid (C18:1), α-linolenic acid (C18:3 n-3), γ-linolenic (C18:3 n-6), behenic acid (C22:0), and arachidonic acid (C20:4 n-6) FAs ($P < 0.05$; Table 1).

The highest CLA was found in ST muscle type (0.298%) and LW (0.289) group in Anatolian buffaloes ($P > 0.05$; Table 1). In the study, the differences between SW groups respecting n-6 and between MT groups respecting n-6/n-3 ratio were found significant ($P < 0.05$; Fig. 2). The PUFA ratio was determined higher in ST muscle than SM and TB muscles ($P > 0.05$) and higher in the LW group than the HW group ($P < 0.05$; Table 1). In terms of SFA, the differences between the SW groups were insignificant ($P > 0.05$), while the differences observed between MTs (ST, SM, and TB) were significant ($P < 0.05$; Table 1). PUFA/SFA ratio decreased in Anatolian buffaloes with increasing SW ($P < 0.05$; Table 1; Fig. 2). The lowest AI (0.510) and TI (1.488) values were found in the LW group ($P > 0.05$). In the study, the TI value was higher in TB muscle than in SM muscle ($P < 0.05$; Table 1). In terms of NV and DFAs values, the differences observed respecting MTs and SW groups were found insignificant ($P > 0.05$), but an increase in SW increased NV and DFA values in Anatolian buffaloes. In the study, the C20:4 n-6 ratio decreased with increasing SW in the SM, ST, and TB muscles ($P < 0.05$; Table 2). No significant differences were found in TB muscle in the SW groups (LW and HW) in terms of C22:0 ($P > 0.05$; Table 2). While SW increases, the SFA ratio increased in the SM and ST muscles but decreased in the TB muscle ($P < 0.05$; Fig. 1[x]). Again with the increase in SW, AI and TI values increased in SM and ST muscles, while the index values decreased in TB muscle ($P > 0.05$).

Comparing all MTs and SW groups revealed that the lowest CLAs, n-3, n-6, n-6/n-3, and PUFA/SFA ratios were in the HW group (Table 1; Fig. 2). In the present study, with the increase in SW in all muscle groups, NV and DFA values increased (Table 2). Interactions between SWs and MTs were insignificant ($P > 0.05$).

Discussion

CLA from linoleic C18:2 and C18:3 acids found in the ruminants' diet has many geometric and functional isomers. Although there are many geometric isomers of C18:2, it has been reported that mainly cis-9, trans-11, and trans-10, cis-12 forms exist in the natural environment (Banni 2002; Gonçalves et al. 2010). Some studies report that CLA has immunomodulatory, anticarcinogenic, and anti-arteriosclerosis properties (Whigham et al. 2000; den Hartigh, 2019; Basak and Duttaroy, 2020). In the study, CLA was determined on these isomers in Anatolian buffalos. The study found that the highest CLA was in the ST muscle ($P > 0.05$; Table 1). Similarly, Kundi buffaloes (KB) (Talpur et al. 2007) were reported to have the highest CLA content in the ST muscle. The CLAs, which have been emphasized because of their benefits on human health in recent years, existed abundantly in the ST muscle. In studies conducted on young Italian Mediterranean buffalo bulls (Cutrignelli et al. 2013; Calabrò

Table 1 The effect of muscle type and slaughter weight on conjugated linoleic acid and tissue fatty acid composition (wt. %)

Fatty acids	Muscle (MT)			Slaughter weight (SW)		SE	P	
	SM	ST	TB	LW	HW		MT	SW
C14:0	1.060	1.129	1.117	1.057	1.148	0.027		
C16:0	23.190	22.880	22.380	22.910	22.930	0.214		
C16:1	1.771^{a}	1.227^{b}	1.243^{b}	1.354	1.474	0.908	*	
C18:0	19.990^{b}	22.050^{a}	23.300^{a}	21.600	21.96	0.314	***	
C18:1	40.220	38.390	38.110	37.780^{b}	40.040^{a}	0.516		*
C18:2 n-6	7.538	7.856	7.796	8.252	7.208	0.330		
C18:3 n-3	0.732^{a}	0.708^{a}	0.689^{b}	0.782^{a}	0.636^{b}	0.029	**	*
C18:3 n-6	0.203^{a}	0.163^{b}	0.175^{b}	0.210^{a}	0.150^{b}	0.014	**	*
C20:0	0.277	0.227	0.332	0.245	0.316	0.040		
C20:1	0.140	0.106	0.080	0.098	0.119	0.021		
C20:2 n-6	0.293^{c}	0.476^{a}	0.353^{b}	0.454	0.294	0.045	*	
C20:3 n-3	0.957^{a}	0.764^{b}	0.690^{c}	0.827	0.780	0.064	*	
C20:4 n-6	2.959^{b}	3.285^{a}	3.125^{a}	3.635^{a}	2.611^{b}	0.160	**	**
C22:0	0.459^{a}	0.439^{a}	0.366^{b}	0.509^{a}	0.334^{b}	0.026	***	**
CLAs	0.200^{c}	0.298^{a}	0.238^{b}	0.289	0.201	0.029	*	
n-3	1.689	1.472	1.380	1.610	1.418	0.079		
n-6	10.990	11.780	11.450	12.550^{a}	10.260^{b}	0.482		*
SFA	44.980^{b}	46.730^{ab}	47.500^{a}	46.320	46.490	0.364	*	
MUFA	42.130^{a}	39.720^{b}	39.430^{b}	39.230^{b}	41.630^{a}	0.511	*	*
PUFA	12.890	13.550	13.070	14.450^{a}	11.890^{b}	0.544		*
UFA	55.020^{a}	53.270^{ab}	52.500^{b}	53.680	53.510	0.364	*	
MUFA/SFA	0.936	0.849	0.830	0.846	0.895	0.437		
AI	0.503	0.519	0.515	0.510	0.514	0.018		
TI	1.410^{b}	1.532^{ab}	1.582^{a}	1.488	1.528	0.026	*	
NV	2.611	2.653	2.756	2.609	2.738	0.039		
DFA	75.010	75.320	75.800	75.280	75.470	0.255		

LW low slaughter weight, *HW* high slaughter weight

SM semimembranosu, *ST* semitendinosus, *TB* triceps brachii

SFA total saturated fatty acids, *MUFA* total monounsaturated fatty acids, *PUFA* total polyunsaturated fatty acids, *UFA* total unsaturated fatty acids, *DFA* total desirable fatty acids, *NV* nutritive value, *AI* atherogenic index, *TI* thrombogenic index, *CLAs* conjugated linoleic acids (cis9-trans11 CLA + trans10-cis12 CLA)

$^{a, b}$The observed differences between the mean denoted by different letters in the same row are significant

$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

et al. 2014), the CLA content of ST muscle was higher than that of LT and IP muscles. Contrary to these research findings, a study examining the Italian Mediterranean buffaloes' LD, TB, and SM muscles (Romano et al. 2007) determined the highest CLA content in the SM muscle. The CLA differences between MTs were significant and consistent with the results of some studies (Romano et al. 2007; Talpur et al. 2007; Cutrignelli et al. 2013; Calabrò et al. 2014). It has been reported that this situation may be due to potential tissue differences as well as the expression or activity of Δ^9 desaturase enzyme (Garcia et al. 2008; Serra et al. 2009). The study found the CLA content detected in the ST muscle greater than the findings of some studies (Cutrignelli et al. 2013; Calabrò et al. 2014). Examining the SW groups' CLA contents showed that the lowest CLA content was in the HW group's SM muscle (0.185) and the highest in the LW group's ST muscle (0.368) ($P < 0.05$; Table 2). The study determined that as SW increased in LW and HW groups, CLA in SM, ST, and TB muscles decreased ($P < 0.05$). The CLA content determined in the HW group's ST muscle was lower than the values found in the Mediterranean buffaloes' ST muscle and higher than the values detected in the LT and IP muscles of Mediterranean buffaloes (Cutrignelli et al. 2013; Calabrò et al. 2014). The CLA content determined for SW groups was lower than the Kundi buffalos' findings (Talpur et al. 2007) at 392 kg SW. The study found that the CLA content decreased as the SW increased. Likewise, research of cattle (Moreno et al. 2008) reported that as SW increased, the CLA content decreased. This consequence shows that the decrease in CLA content with the increase in

Table 2 Conjugated linoleic acid and tissue fatty acid composition according to muscle type and slaughter weight (wt. (%))

Fatty acids	SM		ST		TB		SE
	LW	HW	LW	HW	LW	HW	
C14:0	0.987[b]	1.137[a]	1.066[b]	1.190[a]	1.114	1.119	0.121
C16:0	23.234	23.155	22.893	22.856	22.590	22.178	0.530
C16:1	1.559[b]	2.002[a]	1.241	1.221	1.270	1.216	0.205
C18:0	19.778	20.209	21.193	22.833	23.762	22.838	0.597
C18:1	38.952	41.506	37.441	39.356	36.956	39.262	1.078
C18:2 n-6	8.359	6.680	8.472	7.251	7.936	7657	0.816
C18:3 n-3	0.821	0.641	0.787	0.629	0.740	0.638	0.069
C18:3 n-6	0.257[a]	0.146[b]	0.182[a]	0.142[b]	0.191[a]	0.158[b]	0.030
C20:0	0.236	0.319	0.223	0.235	0.270	0.394	0.100
C20:1	0.080	0.199	0.144	0.074	0.068	0.091	0.040
C20:2 n-6	0.361[a]	0.226[b]	0.579[a]	0.375[b]	0.424[a]	0.283[b]	0.035
C20:3 n-3	0.954	0.963	0.850	0.685	0.684	0.696	0.132
C20:4 n-6	3.629[a]	2.268[b]	3.403[a]	2.988[b]	3.792[a]	2.593[b]	0.368
C22:0	0.564[a]	0.353[b]	0.557[a]	0.324[b]	1.270	1.216	0.056
CLAs	0.218[a]	0.185[b]	0.368[a]	0.231[b]	0.285[a]	0.191[b]	0.101
n-3	1.775 [a]	1.605[b]	1.637[a]	1.314[b]	1.424[a]	1.335[b]	0.025
n-6	12.606[a]	9.321[b]	13.234[a]	10.362[b]	11.851[a]	11.049[b]	1.148
n-6/ n-3	7.783[a]	6.533[b]	8.115[a]	7.859[b]	8.416[a]	8.215 [b]	0.415
MUFA	40.599	43.706	38.826	40.652	38.295	40.570	1.158
PUFA	14.600[a]	11.119[b]	15.240[a]	11.907[b]	13.561[a]	12.576[b]	1.109
UFA	55.119	54.826	54.067	52.559	51.856	53.149	1.010
AI	0.496	0.510	0.507	0.530	0.526	0.504	0.020
TI	1.383	1.437	1.457	1.600	1.617	1.546	0.059
NV	2.547	2.674	2.573	2.731	2.705	2.807	0.095
DFA	74.977	75.035	75.260	75.393	75.617	75.984	0.565

LW low slaughter weight, *HW* high slaughter weight

SM semimembranosus, *ST semitendinosus*, *TB triceps brachii*

SFA total saturated fatty acids, *MUFA* total monounsaturated fatty acids, *PUFA* total polyunsaturated fatty acids, *UFA* total unsaturated fatty acids, *DFA* total desirable fatty acids, *NV* nutritive value, *AI* atherogenic index, *TI* thrombogenic index, *CLAs* conjugated linoleic acids (cis9-trans11 CLA + trans10-cis12 CLA)

[a,b]The observed differences between the mean denoted by different letters in the same row are significant. $P < 0.05$

slaughter weight, the increase in other fatty acids is higher than the increase in CLA. A similar statement was made by Moreno et al. (2008).

The current study determined that the C14:0 contents of SM, ST, and TB muscles were 1.060, 1.129, and 1.117, respectively, and that muscle type didn't affect ($P > 0.05$) the C14:0 content (Table 1). Calabrò et al. (2014) reported that this effect was significant, unlike our research findings. A study on Italian MB (Calabrò et al. 2014) reported the C14:0 contents of LT, ST, and IP muscles as 1.31, 0.91, and 1.40, respectively. The study found the Murrah buffaloes' C14:0 contents in ST muscle (Sharma et al. 1986) higher than the values of Mediterranean buffaloes (Calabrò et al. 2014). The current research found the C14:0 acid content in SM and TB muscles lower than the Murrah buffaloes' PM muscle and higher than their BF muscle (Sharma et al. 1986). The current research determined that as the SW increased, the C14:0 content in the SM, ST, and TB muscles increased and that the slaughter weight affected the C14:0 content at SM and ST muscles ($P < 0.05$; Table 2). LW and HW groups' C14:0 contents by muscle types were, respectively, as follows: SM muscle 0.987, 1.137; ST muscle 1.066, 1.190; TB muscle 1.114, 1.119. The present study found that C14:0 content in the LW group's ST muscle was similar to Murrah buffaloes reported by Sharma et al. (1986). The C14:0 content in the HW group ST muscle was higher than the Italian Mediterranean buffaloes examined by Calabrò et al. (2014).

It has been reported that C12:0, C14:0 and C16:0, which make up two-thirds of the SFAs content of the diet, increase blood cholesterol levels, while stearic acid (C18:0) does not affect blood cholesterol levels (Denke and Grundy 1992; Zock et al. 1994; Grundy 1997; Yakan and Ünal 2010; Lukic

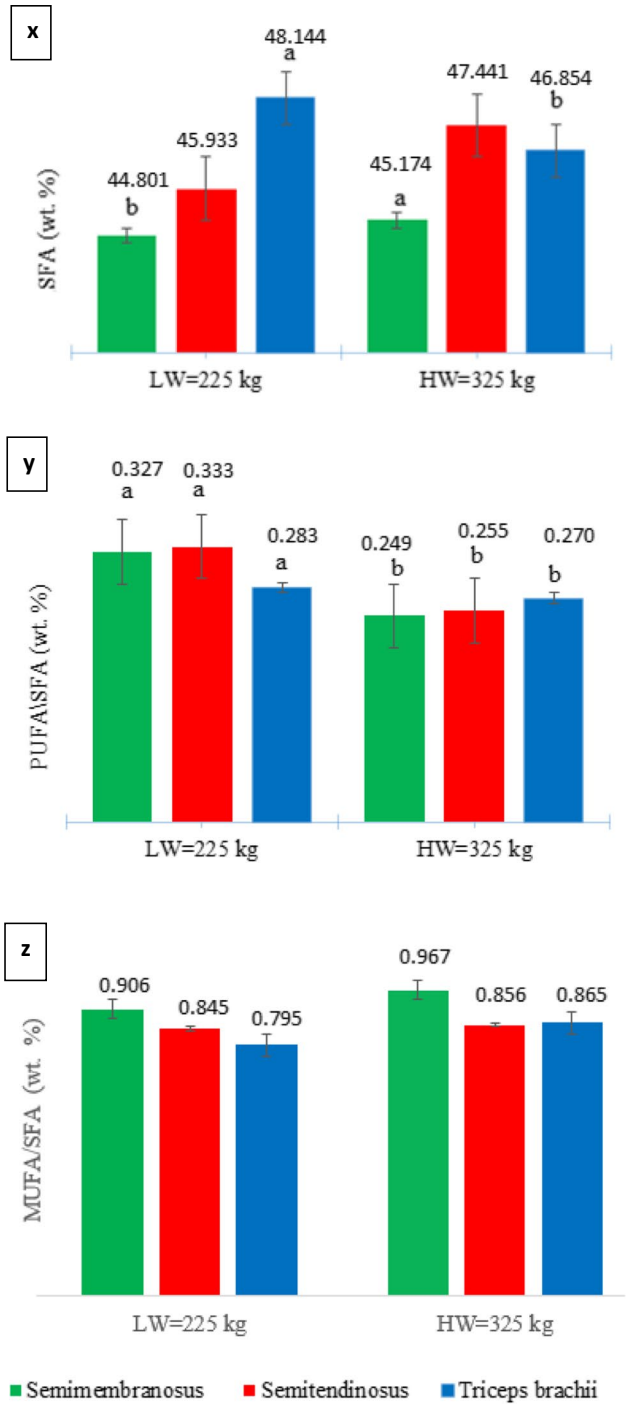

Fig. 1 The effect of slaughter weight on saturated fatty acids (SFA) [x], polyunsaturated fatty acids (PUFA)/saturated fatty acids (SFA) [y], and monounsaturated fatty acids (MUFA)/saturated fatty acids (SFA) [z] content of different muscle tissue in Anatolian buffaloes. The error bars represent the standard error of the mean. a, b: The differences observed between the means shown with different letters in the same colored columns are significant ($P < 0.05$)

et al. 2019; Chen and Liu 2020). The study determined that the C16:0 content was lowest in the TB muscle (22.380) and highest in the SM muscle (23.190) ($P > 0.05$; Table 1). C16:0 content determined in ST muscle was higher than the finding of Calabrò et al. (2014) and lower than the values found in some studies (Sharma et al. 1986; Luz et al. 2017). The differences between LW and HW groups in terms of C16:0 content were insignificant ($P > 0.05$; Table 1). C16:0 was 22.910 in the LW group and 22.730 in the HW group ($P > 0.05$; Table 1). The study found that as SW increased, C16:0 content decreased ($P > 0.05$; Table 2). In male Bulgarian Murrah buffaloes slaughtered at 450 kg live weight and females at 580-600 kg live weight, C16:0 content was determined as 25.75% and 27.81%, respectively (Dimov et al. 2012). C16:0 content detected in this study was lower than the findings of Dimov et al. (2012) and higher than the values reported for Kundi buffaloes (Talpur et al. 2007).

The majority of SFAs in Murrah (Sharma et al. 1986) and Anatolian buffalo (Aksoy et al. 2021) red meats have been reported to be C16:0 and C18:0. Similarly, in this study, C16:0 and C18:0 contents in SW and MT groups were found higher than other FAs. C16:0 was higher in the SM muscle and LW group, while C16:0 was lower in the TB muscle and HW group ($P > 0.05$; Table 2). In this study, while the C16:0 content detected in muscle groups and SW groups was found lower than the findings of some studies (Romano et al. 2007; Dimov et al. 2012; Ilavarasan et al. 2016), it was higher than Kundi buffalo values determined by Talpur et al. (2007) and Anatolian buffalo values by Ekiz et al. (2018). The values were compatible with the Anatolian water buffalo findings of Aksoy et al. (2021). In the study, the C16:0 content in the LW group's ST muscle was similar to the value detected in the Murrah buffaloes' ST, psoas major (PM) muscles, but lower than the values determined for the biceps femoris (BF) and longissimus dorsi (LD) muscles (Sharma et al. 1986). C16:0 content detected in ST, SM, and TB muscles of the HW group was higher than the values detected by Calabrò et al. (2014) in LT, ST, and IP muscles. In the study, the C18:0 value was higher than the Murrah buffaloes' ST muscle value (Sharma et al. 1986) and lower than the PM, LT, and BF muscles, and higher than the Italian Mediterranean buffaloes' ST muscle value found by Calabrò et al. (2014).

Stearic acid is crucial for unsaturated fatty acid synthesis (Mottin et al. 2020). The formation of a double bond between the 9th and 10th carbon atoms is catalyzed by the enzyme delta 9 desaturase. This enzyme is found in plants and animals and plays a role in the conversion of C18:0 to C18:1n-9 (Lee et al. 2016). C14:0 and C16:0 fatty acids are considered hypercholesterolemic and are responsible for increasing LDL leading to heart disease.

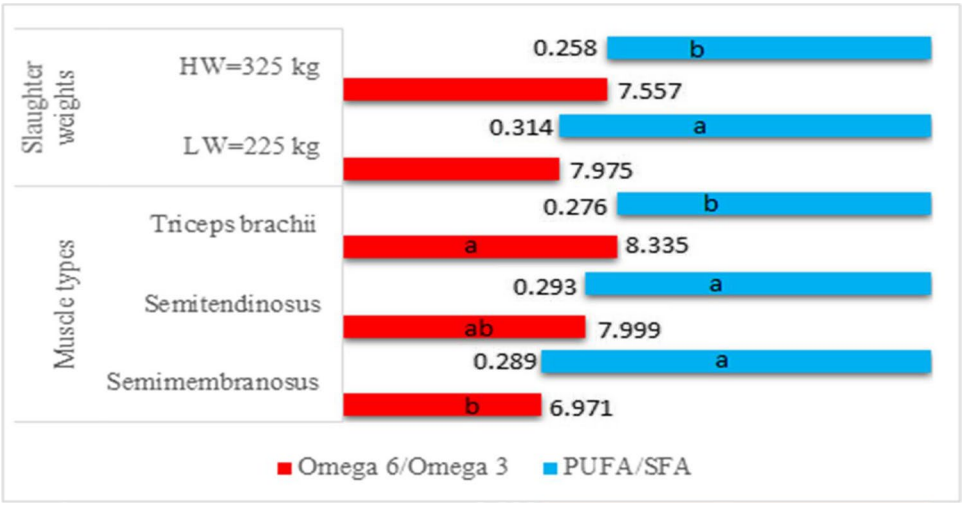

Fig. 2 Variation of polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) and omega 6/omega 3 (n-6/n-3) fatty acids content according to muscle types and slaughter weights in Anatolian buffaloes. a, b: The differences observed between the means shown with different letters on the horizontal bars within the slaughter weight and muscle type groups are significant ($P < 0.05$)

In the present study, with SW increased, C18:0 content increased in ST and SM muscles and decreased in TB muscle ($P > 0.05$; Table 2). The C18:0 content increased with increasing SW ($P > 0.05$; Table 1). This may be due to Δ^9-desaturase activity, as the Δ^9-desaturase enzyme uses fatty acids as a substrate to convert C18:0 to C18:1n-9 (Hirata et al. 2019). The C18:0 content determined in the HW group's ST muscle was higher than the Mediterranean buffalos' ST muscle (Calabrò et al. 2014) and lower than the IP and LT muscle values. The C18:0 content detected in the LW group's SM, ST, and TB muscles was higher than the Murrah breed buffalo's ST muscle values (Sharma et al. 1986) and lower than the values reported for the PM, LD, and BF muscles. The C18:0 content determined in the study was higher than some research results (Dimov et al. 2012; Ekiz et al. 2018) and lower than the values reported for Toda buffaloes (Ilavarasan et al. 2016), Kundi buffaloes (Talpur et al. 2007), and female Murrah buffaloes (Luz et al. 2017). Research findings were compatible with Romano et al.'s (2007) findings determined for the Italian Mediterranean buffaloes' LD and TB muscles and higher than the SM muscle value. SW was determined to have no statistical effect on the contents of C16:0 and C18:0, which was consistent with the finding of a study (Aksoy et al. 2021). Since saturation will increase with increasing slaughter age, comparing low body weight bulls and high body weight bulls reveals that the rise in saturation will be notably related to C18:0 and C20:0 fatty acids (Wood et al. 2008; Indurain et al. 2010). The present work evidenced that the C16:0 ratio was not affected by SW ($P > 0.05$; Table 1). Unlike the current findings, a previous study determined that this effect was significant (Indurain et al. 2010) and might occur with high ruminal activity because of more saturated-fat-inducing high body weight.

In addition, due to the negative relationship between elongase enzyme activity and intramuscular fatty acid (IMF) content, it has been reported that the higher the IMF percentage, the lower the conversion of C16:0 to C18:0 (Kazala et al. 1999; Pitchford et al. 2002; Indurain et al. 2010).

MUFAs have been reported to influence the blood cholesterol level in humans hardly. C18:1 MUFA determines the nutritional value of meat, decreases blood LDL level, and increases high-density lipoprotein (HDL) level (KrisEtherton 1999; Caneque et al. 2001; Yakan and Ünal 2010; Smith et al. 2020). Oleic acid, linolenic acid, and linoleic acid (Hall et al. 2016), which are the precursors of PUFAs in meat, cannot be synthesized by the ruminant's body and therefore must be taken from the diet (Gogus and Smith 2010). In the study, C18:1 content in ST, SM, and TB muscles varied between 38.110 and40.220 ($P > 0.05$; Table 1). The highest C18:1 content was in SM muscle (40.220), which was higher than the values reported for Mediterranean buffaloes (Calabrò et al. 2014). The C18:1 content in the ST muscle was higher than the value in the PM, LT, ST, and BF muscles of Murrah buffaloes (Sharma et al. 1986). It has been reported that oleic acid (C18:1) is found in high concentrations in the intramuscular fat of ruminant animals and is synthesized from stearic acid using the Δ^9-desaturase enzyme (Wood et al. 2008). The study determined that, as SW increased, C18:1 content increased, and C18:1 content was affected by SW ($P < 0.05$). Likewise, a study (Aksoy et al. 2021) reported that SW affected the C18:1 content, and as the SW increased, the C18:1 content increased. It is desirable that the oleic acid content is high. In this study, it can be said that more desirable meat was obtained from animals with a higher slaughter weight than those with a lower slaughter weight, since the oleic acid content increased as the slaughter weight increased. The HW group's C18:1 content detected in the study was consistent with the value determined for Murrah buffaloes (Dimov et al. 2012) at 450 kg SW. In the study, the C18:1 content determined for MTs and SW groups was higher than the values reported for

Kundi (Talpur et al. 2007) buffaloes. In this study, C18:1 content detected in ST, TB, and SM muscles for LW and HW groups was higher than the value in PM, LT, ST, and BF muscles of Murrah buffaloes (Sharma et al. 1986). The C18:1 content determined in the HW group's ST muscle was higher than the values detected in the LT, ST, and IP muscles of Italian Mediterranean buffalos (Calabrò et al. 2014). The C18:1 content found in ST, SM, and TB muscles in LW and HW groups was similar to Dimov et al. (2012) findings, and the SW groups' C18:1 contents were consistent with the values reported for young and adult Toda buffalos (Ilavarasan et al. 2016).

The present study determined C18:2n-6c contents in LW and HW groups as follows: TB muscle 7.936, 7.657; SM muscle 8.359, 6.680; ST muscle 8.472, 7.251, respectively ($P > 0.05$; Table 2).

This study determined that, as SW increased, C18:2n-6c was decreased ($P > 0.05$; Table 1). It can be said that the biohydrogenation process in the rumen plays a role in this situation. Because in this process, PUFAs are converted into SFAs as a way to prevent toxicity from rumen bacteria. After this process, SFAs are absorbed and incorporated into muscle tissue. The C18:2n-6c contents of TB, SM, and ST muscles in both SW groups decreased as the SW increased ($P < 0.05$; Table 2). Results were similar in LT muscle in cattle (Mottin et al. 2020) and Anatolian buffaloes (Aksoy et al. 2021). The present study determined that the LW group ST and SM muscle C18:2n-6c contents were similar to Murrah buffaloes' ST muscle (Sharma et al. 1986); LW group TB muscle C18:2n-6c acid content was close to Murrah buffaloe PM and LT muscles, but it was lower than the value of the BF muscle (Sharma et al. 1986). The HW group ST muscle content was lower than ST and IP muscle values determined by Calabrò et al. (2014) and higher than the LT muscle value.

As for the muscle types, the current study determined the C18:2n-6c content in SM, ST, and TB muscles as 7.538, 7.856, and 7.796, respectively ($P > 0.05$; Table 1). These values were lower than the values determined by Calabrò et al. (2014) for ST and IP muscles and higher than LT muscle. Contrary to research findings, a study (Calabrò et al. 2014) found muscle type affected C18:2n-6c content. The present study found the C18:2n-6c contents in ST, SM, and TB muscles lower than the Murrah buffaloes' ST and BF muscles and higher than the PS and LT muscles.

In SM, ST, and TB muscles, C18:3n-3 content was 0.732, 0.708, and 0.689; C18:3n-6 content was 0.203, 0.163, and 0.175; C20:4n-6 content was 2.959, 3.285, and 3.125; and C22:0 content was 0.459, 0.439, and 0.366. Muscle types affected these FAs ($P < 0.01$; $P < 0.001$; Table 1). C18:3n-3, C18:3n-6, C20:4n-6, and C22:0 contents in SM and TB muscles were higher than the findings of Calabrò et al. (2014), and in ST muscle was similar to them.

C18:3n-6, C20:4n-6, and C22:0 contents were 0.257, 3.629, and 0.564 in LW group SM muscle; 0.182, 3.403, and 0.557 in ST muscle; 0.191, 3.792, and 1.270 in TB muscle, respectively (Table 2). C18:3n-6, C20:4n-6, and C22:0 contents were 0.146, 2.268, and 0.353 in HW group SM muscle; 0.142, 2.988, and 0.324 in ST muscle; 0.158, 2.593, and 1.216 in TB muscle, respectively ($P < 0.05$; Table 2). C18:3n-6 content determined in HW group ST muscle was similar to the ST muscle value determined by Calabrò et al. (2014), but it was higher than the value found in the LT and IP muscles. C20:4n-6 contents determined in HW group ST, SM, and TB muscles were lower than the ST muscle value found by Calabrò et al. (2014), but they were higher than the values found in the LT and IP muscles. The C20:4n-6 contents in the ST, SM, and TB muscles of the LW group were similar to the value detected in the Murrah breed buffaloes' ST muscle (Sharma et al. 1986), higher than the values detected in the PM and LD muscles and lower than the value detected in the BF muscle. C22:0 content in HW group ST, SM, and TB muscles was higher than the LT, ST, and IP muscles values determined by Calabrò et al. (2014). The effect of SW on C18:3n-6, C20:4n-6, and C22:0 contents was significant ($P < 0.05$; Table 2). The current study determined that C18:3n-3, C18:3n-6, C20:4n-6, and C22:0 decreased as SW increased in Anatolian buffaloes' SM, ST, and TB muscles.

The study determined that C20:0 content was 0.277, 0.227, and 0.332 in SM, ST, and TB muscles, respectively, and that muscle type did not affect C20:0 content ($P > 0.05$; Table 1). Contrary to the research finding, Calabrò et al. (2014) reported that C20:0 content was affected by muscle type. The C20:0 content in the ST muscle was higher than the Italian Mediterranean buffaloes (Calabrò et al. 2014). The C20:0 contents in the LW and HW groups, respectively, were found as follows: SM muscle 0.236 and 0.319; ST muscle 0.223 and 0.235; TB muscle 0.270 and 0.394. The slaughter weight did not affect the C20:0 content ($P > 0.05$; Table 2). The current study observed that C20:0 content for HW group ST muscle was higher than the Italian Mediterranean buffaloes observed by Calabrò et al. (2014). The C20:0 contents detected in the ST, SM, and TB muscles of the LW group were lower than the value found in the Murrah buffaloes' PM muscle but higher than their BF muscle (Sharma et al. 1986).

With increasing SW, C20:1 and C20:3n-3 contents increased in SM and TB muscles and decreased in ST muscle. The current study determined that the C20:1 content of LW and HW groups was 0.080 and 0.199 in SM muscle, 0.144 and 0.07 in ST muscle, and 0.068 and 0.091 in TB muscle ($P > 0.05$; Table 2). The C20:1 content in the ST muscle of the HW group was lower than the value in the IP and LT muscles of Mediterranean buffalo bulls (Calabrò et al. 2014). C20:1 content was determined as 0.140, 0.106,

and 0.080 in SM, ST, and TB muscles, respectively. The effect of muscle type on C20:1 content was insignificant ($P > 0.05$; Table 1). This result was inconsistent with the finding of Calabrò et al. (2014). In the study, the C20:1 contents in SM and ST muscles were similar to the value detected in ST muscle of Italian Mediterranean buffalo bulls but higher than the value detected in LT and IP muscles. C20:1 content determined in TB muscle was consistent with the IP muscle value found by Calabrò et al. (2014). C20:3n-3 contents in LW and HW groups were 0.850 and 0.685 in ST muscle, 0.954 and 0.963 in SM muscle, and 0.684 and 0.696 in TB muscle ($P > 0.05$; Table 2). With rising SW, C20:3n-3 content increased in SM and TB muscles and decreased in ST muscle ($P > 0.05$; Table 2). C20:3n-3 content was 0.957 in SM muscle, 0.764 in ST muscle, and 0.690 in TB muscle ($P < 0.05$; Table 1). In the study, C20:3n-3 content in ST, SM, and ST muscles was higher than the Italian Mediterranean buffalo LT, ST, and IP muscle values detected by Calabrò et al. (2014). The muscle type affected the C20:3n-3 content ($P < 0.05$; Table 1), and this result was compatible with the findings of a study (Calabrò et al. 2014). C20:3n-3 contents in Italian Mediterranean buffaloe LT, SP, and IP muscles were 0.23, 0.33, and 0.28, respectively ($P > 0.05$) (Calabrò et al. 2014). The current study revealed the C20:3n-3 content in the HW group ST, SM, and TB muscles higher than these reports. SW was determined to have no impact on the C20:3n-3 content ($P > 0.05$; Table 1). Contrary to the research findings, a study (Calabrò et al. 2014) found this effect significant. The research finding was different from this report.

Eicosadienoic acid (C20:2n-6) contents were 0.293 in SM muscle, 0.476 in ST muscle, and 0.353 in TB muscle ($P < 0.05$; Table 1). The highest eicosadienoic acid content value was in ST muscle and the smallest value in SM muscle. The study by Calabrò et al. (2014) also found a similar result and reported that eicosadienoic acid content was affected by muscle type, as in the current study. On the other hand, the contents of eicosadienoic acid were 0.18, 0.26, and 0.18 in the LT, ST, and IP muscles of Italian Mediterranean buffaloes, respectively (Calabrò et al. 2014). The research finding was higher than these reports. Eicosadienoic acid contents were 0.424 and 0.283 in TB muscle, 0.579 and 0.375 in ST muscle, and 0.361 and 0.226 in SM muscle in LW and HW groups ($P < 0.05$; Table 2). In the study, eicosadienoic acid content in LW and HW groups ST, TB, and SM muscles decreased as SW increased ($P < 0.05$; Table 2).

In the current research, eicosadienoic acid content in TB and ST muscle of LW and HW groups was higher than the Mediterranean buffaloe ST muscle value reported by Calabrò et al. (2014). Eicosadienoic acid content detected in SM muscle was higher than the LW group ST muscle found by Calabrò et al. (2014) but lower than the value of the HW group.

Eicosadienoic acid contents in LW group ST, SM, and TB muscles were lower than the ST but similar to BP muscle value determined by Sharma et al. (1986). Eicosadienoic acid contents in LW group SM muscle were lower than the PM and LD muscle values observed by Sharma et al. (1986).

The meat of ruminants contains more SFA than monogastric because of hydrogenates in the rumen (Wood et al. 1999). SFAs have been reported to increase the progression, rate, and risk of multiple sclerosis (MS), but PUFAs have beneficial effects for MS patients (Chen and Lui 2020; Langley et al. 2020). The study found the differences between MTs as significant, respecting the SFA ratio ($P < 0.05$; Table 1). In a study with similar findings (Calabrò et al. 2014), MT was found to affect the rate of SFA. In the study, the SFA values in ST, SM, and TB muscles were higher than the values in the Italian Mediterranean buffalo bulls' ST muscles and lower than the LT and IP muscles (Calabrò et al. 2014).

The study determined SFA values in LW and HW groups as TB: 48.144 and 46.854, SM: 44.801 and 45.174, and ST: 45.933 and 47.441, respectively (Fig. 1[x]; $P < 0.05$). With rising SW, the SFA content was determined to increase in the SM and ST muscles but decrease in the TB muscle. In the study, the effect of SW on the SFA was significant ($P < 0.05$; Table 2), which was compatible with the finding of Aksoy et al. (2021). SFA content slightly was increased as slaughter weight increased (Table1; $P > 0.05$). This observation can be attributed to the hydrogenation carried out by microorganisms. In the study, the HW group's SFA value for ST, SM, and TB was higher than the Italian Mediterranean buffaloes' ST muscle value and lower than the LT and IP muscles (52.5 and 53.5) (Calabrò et al. 2014). In the study, the SFA values in the LW group's ST and TB muscles were higher than those reported for Murrah buffaloes and were similar to the value of SM muscle (Sharma et al. 1986). The study found the SFA value in the TB muscle higher than the value reported by Sharma et al. (1986) for the Murrah buffaloes' ST muscle and lower than PM, LT, and BF muscle values. In the study, the SFA contents detected in SM, ST, and TB muscles were higher than the SFA value in the LD muscle of Mediterranean buffaloes (Infascelli et al. 2005), but it was lower than the LT muscle values of Anatolian buffaloes (Aksoy et al. 2021) slaughtered at 200 kg live weight. The SFA value found in the study was consistent with the finding of Dimov et al. (2012), lower than the values determined for KB (Talpur et al. 2007) and Murrah (Rebak et al. 2010) buffaloes and higher than the value declared for Italian MB (Infascelli et al. 2005). In the study, the SFA values for the LW and HW groups were determined as 46.320 and 46.490, respectively. This result was lower than the reported values for young and adult Toda buffaloes (Ilavarasan et al. 2016) and Italian Mediterranean buffaloes (Romano et al. 2007). Due to its positive effects

on human health in diets, a high PUFA ratio will keep the PUFA/SFA ratio at desired levels (≥ 0.45). When compared, C18:2n-6, which is the predominant PUFA, is reported to lower LDL cholesterol concentrations slightly more than C18:1. However, it has been reported that extreme levels of C18:2n-6 in the diet will promote chemical carcinogenesis and reduce the immune system (Grundy 1997; Yakan and Ünal 2010; Aksoy et al. 2021; Şahin et al. 2021). In the study, the PUFA value detected in the ST (13.55) muscle of Anatolian buffaloes was consistent with the ST muscle value determined in the Murrah breed bulls (Sharma et al. 1986); it was lower than the value of the Italian Mediterranean buffalo bulls (Calabrò et al. 2014). The study found that the PUFA value detected in the SM muscle was higher than the value reported by Sharma et al. (1986) for the LD and PS muscles of Murrah breed buffalo bulls and lower than the BF muscle value. PUFA value detected in TB muscle in the study was higher than the LT and IP muscle values by Calabrò et al. (2014). PUFA ratio in SM muscle was higher than the of Mediterranean buffaloes' LT muscle value and consistent with the IP muscle value (Calabrò et al. 2014). In the study, the highest PUFA rate was in the LW group's ST muscle ($P < 0.05$; Table 2). The PUFA value detected in the ST muscle of the HW group was lower than the Italian Mediterranean buffaloes' ST muscle observed by Calabrò et al. (2014). The study found that the rate of PUFA in the SM and TB muscles of the LW group was higher than the value of the LT muscle of Italian Mediterranean buffaloes Calabrò et al. (2014) and lower than the values detected in the IP muscle. In the study, the PUFA ratio determined in the LW group's ST muscle was higher than the value observed in the ST muscle of the Murrah breed buffalo (Sharma et al. 1986) slaughtered at 250 kg live weight. The study revealed that the PUFA ratio measured in the SM and TB muscles of the LW group was lower than the values of Murrah buffaloes' BF muscle found by Sharma et al. (1986) and higher than the values found in the PM and LD muscles. In the study, the PUFA contents were 14.450 and 11.890 for the LW and HW groups, respectively, and the PUFA value decreased as the SW increased ($P < 0.05$; Table 1).

This low PUFA content in meat may be related to the biohydrogenation process in which PUFAs are converted to SFAs by bacteria of rumen. This result was also achieved in studies performed in Anatolian buffaloes (Aksoy et al. 2021) and Toda buffaloes (Ilavarasan et al. 2016). Contrary to the research findings, it has been reported that the increase in SW doesn't affect the PUFA ratio in Murrah buffaloes (Dimov et al. 2012). A study conducted on Toda buffaloes has stated that the PUFA contents are 8.23 and 5.66 for young and adult buffaloes, respectively, and the differences between the averages are significant (Ilavarasan et al. 2016). Reportedly, muscles containing large amounts of unsaturated fatty acids (UFA) are more delicious; especially foods containing PUFA are beneficial for human health (Calabrò et al. 2014; Honda et al. 2016; Kohama et al. 2021).

The high n-3 ratio in the diet is substantial to strengthen the immune system, reducing the blood triglyceride level and blood LDL level and reducing the risk of blood coagulation disorders (Larsen 2000; Moreno and Mitjavila 2003; Turan et al. 2013; Aksoy et al. 2021). In the study, the highest n-3 was in the SM muscle (1.689) and the lowest in the TB muscle (1.380) ($P > 0.05$; Table 1). As the SW increased, the n-3 value decreased in the study ($P < 0.05$; Table 2). This situation was similar to the value found in a study on Holstein bulls (Kul et al. 2020). In the study, according to MTs, the highest n-3 ratio was in the LW group (1.424–1.775) (Table 2). The study showed that the n-3 ratio decreased with rising SW in the ST, SM, and TB muscles. In the study, the n-3 ratio determined in the HW group's SM, ST, and TB muscles was lower than the value found in Italian Mediterranean buffaloes slaughtered at 350 kg (Calabrò et al. 2014). The n-3 ratio detected in the SM muscle of the HW group was consistent with the values found in the LT and IP muscles of Italian Mediterranean buffaloes. The n-3 ratio detected in the TB muscle was lower than the value determined in the LT, ST, and IP muscles of Italian MB (Calabrò et al. 2014). In the study, the n-3 values determined for muscle types and SW groups were lower than the values reported for KB (Talpur et al. 2007).

The FA content of meat, especially the n-3 and n-6 families, has many benefits for human health (Simopoulos 2016). n-3 fatty acids, in particular, reportedly play a crucial physiological role in fetal and infant development, shaping the retina and central nervous system (Bourre 2003; Bowen and Clandinin 2005), and are also vital to prevent cardiovascular diseases (Simon et al. 1995; Hu et al. 1999; Mantzioris et al. 2022).

It has been reported that an increase in the n-6 ratio in diets will increase the risk of many diseases such as cardiovascular diseases, cancer and inflammatory diseases (Simopoulos 2008). For this reason, some research suggests that raising n-3 and n-6 intake should be decreased in diets to prevent and manage chronic diseases (Simopoulos 2002). The highest n-6 value was in the ST muscle. In the study, the differences in ST, SM, and TB muscles in terms of n-6 were insignificant ($P > 0.05$), while the differences in the SW groups (LW and HW) were significant ($P < 0.05$; Table 1). Many researchers have reported that a low n-3 ratio and a high n-6 ratio in the diet will increase the n-6/n-3 ratio, and thus, the cardiovascular disease risk ratio will increase (Simopoulos 2008, 2016; Nogalski et al. 2018; Basak and Duttaroy 2020; Kul et al. 2020; Mottin et al. 2020). According to many studies, many types of cancer can be suppressed or prevented with diets with a balanced n-6/n-3 ratio. For this

reason, the ideal rate of n-6/n-3 has been recommended to be 4–5:1 or 1:1 (Gómez Candela et al. 2011; Turan et al. 2013; Aksoy et al. 2021; Şahin et al. 2021), or, in other words, it should not exceed 4 (Anonymous 1994b). It has been documented that excessively high n-6/n-3 content in the diet other than the recommended ratio could increase the risk of advanced prostate cancer (Gómez Candela et al. 2011; Turan et al. 2013). In the study, the LW group's n-6/n-3 ratio varied between 7.783 and 8.416 and the HW group's between 6.533 and 8.215, and the n-6/n-3 ratio decreased with increasing SW in all three MTs ($P < 0.05$; Fig. 2). The study determined that MT affected the n-6/n-3 rate ($P < 0.01$), and this finding was consistent with the research by Calabrò et al. (2014). In the study, the ratio found in the HW group's ST and TB muscles was higher than the values calculated by Calabrò et al. (2014) for LT, ST, and IP muscles. The n-6/n-3 ratio detected in the HW group's SM muscle was higher than the values of the LT and ST muscles and lower than the IP muscle observed by Calabrò et al. (2014).

The current research finding was higher than those regarding the n-6/n-3 ratio reported by Alabiso et al. (2020). However, the optimal nutritional value of the n-6/n-3 ratio has yet to be fully elucidated for both humans and animals. Researchs on the connection between the n-6/n-3 rate and the pathogenesis of many diseases, including cancer, cardiovascular, inflammatory, and autoimmune diseases, show that the optimal level may change according to the situation or illness. This situation is coherent with the fact that chronic ailments are multifactorial and multigenic. Health authorities in many countries usually encourage the intake of foods with a high n-6/n-3 ratio (Meyers and Suitor 2007). This situation reportedly reduces the threat of various chronic ailments (Simopoulos, 2003). In the study, the n-6/n-3 ratios determined for ST, SM, and TB muscles (Fig. 2) and SW groups were higher than the values reported for KB (Talpur et al. 2007).

The PUFA/SFA ratio is an index commonly used to evaluate the effect of diet on cardiovascular health (Chen and Lui 2020). The study observed the highest PUFA/SFA ratio in the ST muscle and the lowest in the TB muscle (Fig. 2). The differences between PUFA/SFA ratios according to MTs were significant ($P < 0.05$). Similarly, Calabrò et al. (2014) have reported that MT affects the PUFA/SFA ratio. The ST muscle PUFA/SFA ratio was lower than the value found in the Italian Mediterranean buffaloes' ST muscle (Calabrò et al. 2014). PUFA/SFA ratio detected in SM and TB muscles was higher than the LT and IP muscle values recorded by Calabrò et al. (2014) and consistent with the 0.2–0.3 value ranges reported by Sharma et al. (1986) for Murrah buffaloes. In the study, the PUFA/SFA ratio determined for all three MTs was lower than the value recommended for healthy diets (≥ 0.45) by the World Health Organization and many researchers (Anonymous 1994a; Yakan and Ünal, 2010; Aksoy et al. 2021). It has been reported that this situation may be associated with the hydrogenation of dietary unsaturated fats in the rumen and the high n-6/n-3 ratio in ruminant meats (Enser et al. 1998; Ilavarasan et al. 2016). Most data on the PUFA/SFA and n-6/n-3 were stated to not comply with these limits, as in most cattle breeds (Sevane et al. 2014; Di Stasio and Brugiapaglia 2021).

The highest PUFA/SFA proportion was in the LW group's ST and SM muscles, and the lowest PUFA/SFA proportion was in the HW group's ST and SM muscles ($P < 0.05$; Fig. 1[y]). The PUFA/SFA proportion determined in the TB muscle of the LW group was consistent with the value in the PM, LD, ST, and BF muscles of the Murrah breed buffaloes. The values detected in the SM and ST muscles of the LW group were higher than the values found in the Murrah breed buffaloes (Sharma et al. 1986). PUFA/SFA ratios detected in the HW group's ST, TB, and SM muscles were lower than the value of Italian Mediterranean buffaloes' ST muscle determined by Calabrò et al. (2014) and higher than the values determined for LT and IP muscles. The study revealed that PUFA/SFA ratio decreased as SW increased, and the effect of SW on this ratio was significant. This reduction may be due to the high degree of biohydrogenation of dietary PUFAs by microorganisms of rumen. The low PUFA/SFA ratio is considered a negative trait as it has a role in promoting cholesterolemia. Therefore, it can be said that the nutritional quality of the meat obtained in the LW group is better. Some studies also found that the PUFA/SFA ratio decreased with increasing SW (Dimov et al. 2012; Aksoy et al. 2021). The PUFA/SFA ratio was determined as 0.10 and 0.13 in adult and young Toda buffaloes (Ilavarasan et al. 2016). This study observed that the PUFA/SFA ratio of MTs and SW groups was higher than the values determined for KB (Talpur et al. 2007) and Toda buffaloes (Ilavarasan et al. 2016).

The current study found high SFA and MUFA rates in the HW group but a low rate of PUFA (Table 1). In the study, MUFA content increased, and PUFA content decreased with increasing slaughter weight (Table 1; Table 2). The increase in MUFA content by the conversion of C18:0 to C18:1n-9 and the decrease in the contribution of PUFA to the fatty acid profile may have caused this situation. A similar result was obtained in a study (Indurain et al. 2010) conducted on local cattle in Spain. With the slaughter weight, the increase in SFA and MUFA and the decrease PUFA content show the importance of NL in TL. Low use for energy production, PUFA, which is the membrane element, is usually stored in phospholipids (De Smet et al. 2004). The increase in slaughter weight of the buffaloes led to increase in C14:0, C16:1, C:18, and C18:1 values (Table 1). Animals with a high SW have a higher percentage of subcutaneous and intramuscular fat in their meat. This high percentage of fat may change the distribution profile. It has been reported

that the amount of UFAs, especially monounsaturated fatty acids (MUFA), is directly related to total carcass fat (Pereira et al. 2012; Mottin et al. 2020). It has been emphasized that the increase in fat content proportionally reduces the incidence of high phospholipids in cell membrane linoleic acid. When SFA and MUFA are compared, PUFA decreases, and thus, the quantity of phospholipids and PUFA could be proportionally higher in low-fat fresh meats (Alabiso et al. 2020).

The MUFA content in muscle's neutral lipids and total bovine fat is affected by age and increasing MUFA/SFA ratio (Wood et al. 2008). In the current research, the highest MUFA/SFA ratio was found in the SM muscle (Table 1; $P > 0.05$). A previous study on cattle (Alabiso et al. 2020) detected the highest MUFA/SFA proportion in the ST muscle. The highest MUFA/SFA proportion was in the HW group SM muscle, and the lowest was in the LW group TB muscle (Fig. 1[z]).

The increasing SFA content may be associated with fattening and other animal-maturity-related physiological processes. Indeed, similar comments were made by Indurain et al. (2010) as well.

MUFA content is strictly associated to the part of C18:1n-9c. This FA is the end produce of the Δ^9 Desaturase-Elongase Enzyme System, which converts SFA to C14:0, C16:0, and C18:0 (Malau-Aduli et al. 1997). However, reportedly, the Δ^9 desaturase enzyme doesn't play a crucial role in IMF creation in ruminants (Vasta et al. 2009). It has been declared that low PUFA in LW bulls is associated with both higher body weight and other age-related physiological factors, such as ruminal biologicalization capacity (Indurain et al. 2010). Reportedly, AI and TI indices can better characterize the plant or animal food health properties with a easy methodology centred on SFA or PUFA/SFA proportion (Ulbricht and Southgate 1991).

The current study discovered that the muscle type did not affect the AI value. However, Calabrò et al. (2014) reported that muscle type affects the AI value. The same research also documented that muscle type affected TI value, and this result agrees with the current study.

It has been expressed that a low AI value reduces the risk of atherosclerosis, and the TI value is an indicator of thrombosis (Vacca et al. 2008; Yakan and Ünal, 2010). Therefore, low AI and TI values affect the nutritional quality of meat positively (Lazzaroni et al. 2009). In the study, the least AI and TI values were observed in the SM (0.503 and 1.410) and LW groups (0.510 and 1.488) (Table 1). Although the differences between the SW groups regarding AI and TI were insignificant, the differences between MTs were significant in terms of TI ($P < 0.05$; Table 1). In the study, AI and TI values calculated for ST muscle were higher than that of ST muscle calculated by Calabrò et al. (2014). SM and TB muscles' AI and TI values were compatible with the values determined for LT and IP muscles in various studies (Cutrignelli et al. 2013; Calabrò et al. 2014). In the research, AI and TI values increased with increasing SW in SM and ST muscles, while these values decreased in TB muscle ($P > 0.05$; Table 2). On the other hand, NV and DFA values increased with raising SW in all muscle groups ($P > 0.05$; Table 2). The study detected the differences in AI, NV, and DFA values within all muscle groups and SW (LW and HW) groups as insignificant ($P > 0.05$; Table 1), while TI value was affected by MT ($P < 0.05$). Italian Mediterranean buffalo bulls' AI and TI values determined for IP and LT muscles (Cutrignelli et al. 2013; Calabrò et al. 2014) were consistent with the research finding. AI and TI values of the ST muscle of the current study were higher than the values of the above studies. The difference in FA rates in the present paper from the values reported by other researchers may be due to the differences in buffalo breed, breeding system, slaughter weight, slaughter age, and MTs considered in the study.

It has been reported that the amount of Δ^9-desaturase 16 enzyme decreases with the increase in slaughter weight. On the contrary to the this situation, it was declared that the amount of Δ^9-desaturase 18 enzyme decreases as SW increases. These enzymes are responsible for double bond conversion on 9 carbons. As a result, low amount of palmitic acid is converted to palmitoleic acid, as the amount of Δ^9-desaturase 16 decreases due to the decrease in slaughter weight.

With the increase in SW, the amount of Δ^9-desaturase 18 enzyme increases, and C18:0 turns into C18:1 as desaturated (Hirata et al. 2019). In all muscle types, C16:0 and C18:0 (in TB muscle) decrease and increase in contents of C16:1 (in SM muscle), and C18:1 and MUFA proportion with an increase in SW can be associated with this situation. The increase in the SFA proportion with the increase in SW and the increase in the PUFA and PUFA/SFA ratio can be explain by the high degree of biohydrogenation of PUFAs by rumen microorganisms.

In this study, fatty acid profiles of ST, SM, and TB muscles commercially important skeletal muscles were investigated in Anatolian buffaloes. The study found significant differences in FA profiles of the three muscles studied, possibly due to two main reasons. The first reason might be that the sample taken was the edible part of the muscle, and the second may be that the distinct muscle types with different roles have different nutrient storages. The metabolic type of muscle (white or red, slow or fast contraction) and the total lipid content of different muscle fibers may be effective in the differentiation of the fatty acids composition between the different muscles. It has been reported that differences in muscle fiber type between muscles are reflected in differences in fatty acid profiles (Wood et al. 2003).

Conclusion

The study investigated CLA content and tissue FA profile of three MTs (SM, ST, and TB) and two SW (LW and HW) groups in Anatolian buffalo male calves, which are expected to contribute more to red meat production in Türkiye in the future. According to the research results, the highest CLA, PUFA, and PUFA/SFA rates in Anatolian buffaloes were in the ST muscle type and the LW slaughtering group. Again, the lowest AI and TI values were in the ST and LW groups. In the study, n-6/n-3 and PUFA/SFA proportions decreased with the increase in SW. The highest C18:3 n-3 value was in the SM and the LW group. The co-evaluation of the LW and HW groups and muscle groups showed that the highest ratio of CLA, PUFA, and PUFA/SFA was determined in the LW group's ST muscle. With the increasing SW value, n-6/n-3, n-3, PUFA, and PUFA/SFA rates decreased in all three MTs. Along with increasing SW, AI and TI values increased in ST and SM muscles and decreased in TB muscles.

The SM muscle n-6/n-3 ratio (6.971), the HW SW group's n-6/n-3 ratio (6.533), and the MTs and SW groups' n-6/n-3 ratio (SM and HW) (7.557) were close to the recommended values for human health. The recommended n-6/n-3 value for human health was mostly observed in the HW group and SM muscle.

Consequently, when considering the positive effects of FAs on human health, the ST group, LW group, and ST and LW groups were prominent in the study. Although this study has contributed to future studies of Anatolian buffaloes, research on different MT and SW combinations must be planned and conducted to reveal this breed's carcass FA profile in more detail.

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Author contribution AŞ, YA, and ZU designed the experiment and wrote the manuscript. AŞ, EU, YA, and KBA collected data. HE, EU, AŞ, and YA performed fatty acid analysis. All authors read and approved the paper.

Data availability Not applicable.

Declarations

Ethics approval The research was authorized by Kırşehir Ahi Evran University Local Animal Ethics Committee.

Conflict of interest The authors declare no competing interests.

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