Züchtungskunde, **96**, (3) S. 203–216, 2024, ISSN 0044-5401

Meat quality, fatty acid profile and genomic insight of Busha cattle in extensive production systems in Serbia

Miloš Marinković[1], Ivan Životić [2], Dušica Ostojić Andrić[1], Jovana Kuveljić[2], Marina Lazarević[1], Maja Živković[2] and Predrag Perišić[3]

Summary

Identification of SNPs and effective genes associated with meat quality traits and fatty acid (FA) profiles can help in the selection of animals with the best potential. In this study, we characterized the meat quality parameters in young Busha bulls for the first time and further performed GWAS analysis for meat quality traits. The fatty acid profile showed that saturated fatty acids were the most abundant in meat and that oleic fatty acid, which is a monounsaturated fatty acid, had the highest content. The correlation analysis showed that the protein content had a negative correlation ($p < 0.05$) with the pH value and a positive correlation ($p < 0.05$) with the content of polyunsaturated fatty acids. GWAS analysis was performed with the BovineSNP50 v3 DNA Analysis BeadChip using the Illumina IscanTM platform. The data were analyzed with PLINK 1.9. Thirteen SNPs were identified with genome wide significant association with muscle fiber diameter (MFD) and one with meat pH. Several candidate genes for MFD have been identified, including *APOD*, *NTMT2* and *ZBTB37*. Candidate SNPs with near suggestive signifficant association with multiple fatty acids level were identified: ARS-BFGL-NGS-118200, Hapmap48202-BTA-118947, BTA-112619-no-rs. *ANGPTL3* could be a potential new candidate gene influencing the FA profile. This study provided valuable insights into the genetics of Busha meat quality traits. The findings from this study could be used for the sustainable exploitation of this autochthonous cattle breed.

Keywords: GWAS, SNP, autochthonous breed, sustainable breeding,

Zusammenfassung

Fleischqualität, Fettsäureprofil und genomische Erkenntnisse von Busha-Rindern unter extensiven Produktionsbedingungen in Serbien

Die Identifizierung von SNPs und effektiven Genen, die mit Fleischqualitätsmerkmalen und Fettsäureprofilen in Verbindung stehen, kann bei der Auswahl von Tieren mit dem besten Potenzial helfen. In dieser Studie wurde zum ersten Mal die Fleischqualitätsparameter bei jungen Busha-Bullen charakterisiert und außerdem eine GWAS-Analyse für Fleischquali-

[1] Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Serbia,
[2] Laboratory for Radiobiology and Molecular Genetics, "Vinca" Institute of Nuclear Sciences, National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia,
[3] University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Serbia; E-mail: majlo1989@gmail.com

tätsmerkmale durchgeführt. Das Fettsäureprofil zeigte, dass gesättigte Fettsäuren im Fleisch am häufigsten vorkommen und dass die oleische Fettsäure, eine einfach ungesättigte Fettsäure, den höchsten Gehalt hatte. Die Korrelationsanalyse zeigte, dass der Proteingehalt eine negative Korrelation ($p < 0,05$) mit dem pH-Wert und eine positive Korrelation ($p < 0,05$) mit dem Gehalt an mehrfach ungesättigten Fettsäuren aufwies. Die GWAS-Analyse wurde mit dem BovineSNP50 v3 DNA Analysis BeadChip unter Verwendung der Illumina IscanTM-Plattform durchgeführt. Die Daten wurden mit PLINK 1.9 analysiert. Es wurden 13 SNPs mit genomweiter signifikanter Assoziation mit dem Muskelgewebefaserdurchmesser (MFD) und einer mit dem pH-Wert des Fleisches identifiziert. Mehrere Kandidatengene für MFD wurden identifiziert, darunter *APOD*, *NTMT2* und *ZBTB37*. Kandidaten-SNPs mit nahezu suggestiver signifikanter Assoziation mit dem Gehalt an mehreren Fettsäuren wurden identifiziert: ARS-BFGL-NGS-118200, Hapmap48202-BTA-118947, BTA-112619-no-rs. *ANGPTL3* könnte ein potentielles neues Kandidatengen sein, das das Fettsäureprofil beeinflusst. Diese Studie lieferte wertvolle Einblicke in die Genetik der Fleischqualitätsmerkmale von Busha. Die Ergebnisse dieser Studie könnten für die nachhaltige Nutzung dieser autochthonen Rinderrasse genutzt werden.

Schlüsselwörter: GWAS, SNP, autochthone Rinderrassen, nachhaltige Zucht,

1 Introduction

Busha is an autochthonous cattle breed from Serbia and a valuable genetic resource. It belongs to a group of primitive short-horned cattle (Bos brachyceros) with a small body size. Cows can reach a weight of 250 to 300 kg and bulls a weight of 350 to 450 kg. This breed is well adapted to extensive production systems in mountainous regions. The focus of these extensive systems is on meat production and therefore it is important to analyze the meat quality traits. Meat quality traits are influenced by both environmental and genetic factors (Zalewska et al., 2021). Many meat traits have been shown to possess heritability potential (Bolormaa et al., 2013). Protein is a key component of meat, as it significantly influences the nutritional value of the product and is a source of essential amino acids (Geletu et al., 2021). Water holding capacity (WHC) and pH are parameters that have a significant impact on meat quality and influence muscle structure, appearance, color and tenderness (López-Pedrouso et al., 2020). The factors that influence the tenderness of the meat also include the diameter and density of the muscle fibers as well as the content of fat and connective tissue in the muscles (Zheng et al., 2018). The fatty acid content of beef is becoming increasingly important, mainly due to its relation to the risk or protection for cardiovascular disease (Briggs et al., 2017) and its effect on sensory properties such as aroma, flavour, juiciness and softness of meat (Listrat et al., 2020). Over the past decade and a half, GWAS has become a valuable tool for identifying novel associations in a hypothesis-free approach to genetics. In addition, the use of SNP maps provides valuable resources for the examination of genetic variability associated with quantitative traits. GWAS is an efficient, high-throughput method suitable for the detection of common variants associated with specific phenotypes (Visscher et al., 2012). Some advantages that SNPs offer are accessibility in very large numbers, presence in coding and non-coding regions, low frequency of errors and easy comparability of results from different studies (Haynes and Latch, 2012). To the authors' knowledge, this is the first study that chacarterizes meat quality parameters in Busha cattle from Serbia and further performs the GWAS on meat quality traits. The aim of this study is to identify SNPs and effective genes associated with meat quality traits and fatty acid profile in the population of Busha from Serbia. These SNPs and genes can be used to identify animals with potential for meat production.

2 Materials and methods

The experiment was conducted at the Institute for Animal Husbandry in Belgrade, Serbia according to strict ethical criteria and approved by the Veterinary Administration of the Ministry of Agriculture, Forestry and Water Management (approval number 323–07–06608/2021–05).

2.1 Samples collection

Rib cutlets from the IX–XI rib area were used to test the meat properties. The meat samples consist of the XI rib with the associated muscles (longissimus dorsi muscle). The rib cutlets from young Busha bulls came from Dimitrovgrad. The average age of the bulls at slaughter was 18 months with a variation of 15 days. The animals were slaughtered at the end of the vegetation period (end of autumn). The live weights of the bulls were around 180 kg with a variation of 10 kg, the average weight of the processed carcasses was 72.5 kg. All bulls in this study came from one farm in Dimitrovgrad with around 300 breeding cows. This farm is currently the largest Busha farm in Serbia with about 550 animals of all ages and is of great importance for the in-situ conservation of this cattle breed. The mountain pastures of Stara planina are characteristic for this region and are located at over 1000 meters above sea level. The animals were reared in the cow–calf system. The calves were weaned at the age of 6 months and the male calves were then separated and introduced to extensive fattening. Feeding during the vegetation period was based on pasture and in the winter period hay from natural meadows was the predominant source of feed with the addition of rye and oat grains. The selection of animals for slaughter was based on the age, body weight and body condition of the bulls. The slaughter took place in an abattoir in Dimitrovgrad, near the farm where the animals came from. Transport from the farm to the abattoir took 30 minutes. The rest time for animals after transportation and before slaughter was 2 hours. The sampling of meat (rib cutlets) took place immediately after the initial processing of the carcass. Samples were transferred to a freezer during transportation to the Institute for Animal Husbandry.

A total of 40 meat samples (one sample per animal) were used for the analysis of meat quality traits and for genetic analysis but one sample was removed from the study during genotyping quality control. Physico-chemical properties of meat, fatty acid profile and correlations between analyzed traits were carried out with the corresponding number of samples. The genomic DNA of 40 samples was extracted with the DNeasy Blood and Tissue Kit according to the manufacturer's instructions (Qiagen, Germany).

2.2 Physico-chemical properties of meat

The following parameters were analyzed when examining the physical and chemical properties of the meat. The protein content was analyzed using the Kjeldahl method (SRPS ISO 937, 1992) on the Kjeltec System 1026 instrument (Foss Tecator, Denmark), and the results were expressed as mass percentage. The pH values were determined using a pH meter with a combined rod electrode Hanna HI 83141 (Hanna Instruments, USA). The water-holding capacity (WHC) was determined according to the method of Grau et al. (1953), whereby the WHC value is expressed in cm^2 of wetted surface. The tenderness of the meat was determined using consistency meter by Volodkevich (1938) by cutting the meat into $0.5 \times 1 \times 2$ cm pieces in the direction of the muscle fiber expansion after cooking at 100°C for 10 minutes. Higher values indicate greater cutting force, suggesting tougher meat, while lower values indicate softer meat.

The following method was used to analyse the fatty acid profile. Fat extraction was performed according to the method of Folch et al. (1957), and the fatty acid methyl esters

were obtained by transesterification with trimethyl sulphur hydroxide (SRPS EN ISO, 5509, 2007). The analysis was performed using a gas chromatograph (Shimadzu – Kyoto, Japan) equipped with a split/splitless injector, an HP-88 column (length 100 m, diameter 0.25 mm, film thickness 0.20 μm) with a cyano-silicone ('fused silica cyanopropyl') stationary phase and a flame ionisation detector (FID). The temperature of the injector evaporator was set to 250°C and that of the detector to 280°C. Nitrogen served as the carrier gas with a flow rate of 1.33 ml/min. The composition of the fatty acids is expressed as the percentage of each fatty acid in the total fatty acids. The diameter of the muscle fibers was measured on a lanometer, with a minimum of 100 measurements per sample. The values obtained with the lanometer were multiplied by a factor of 6.67 to convert the results into micrometers (μm).

2.3 Genotyping and data analysis

The 40 young Busha bulls from Serbia were genotyped with the BovineSNP50 v3 DNA Analysis BeadChip using the Illumina IscanTM platform. The BovineSNP50 BeadChip array features more than 53,000 evenly distributed SNP probes spanning the bovine genome. X-linked SNPs have been omitted from the analysis. Quality control was performed with Plink software version 1.9 beta (46 www.cog-genomics.org/plink1.9). GWAS analyzes were performed using PLINK (Purcell et al., 2007). A linear regression using an additive genetic model was applied, and defined as follows:

$$\mathbf{Y = Xb + Wg + e}$$

Where **Y** was a vector of the trait; **b** was a vector of fixed effect (fat content for fatty acids analysis) and linear discriminant functions; **g** was a vector for the SNP effects; **e** was a random residual effects vector; **X** and **W** were incidence matrices for b and g respectively.

GC correction and False Discovery Rate correction with Benjamini-Hochberg and Benjamini & Yekutieli corrections were also calculated and presented. Samples (animals) and SNPs were excluded from the analysis according to the following criteria: Samples with a call rate < 95%; SNPs with a minimum allelic frequency of less than 0.05; SNPs that were not in Hardy-Weinberg equilibrium (P-value < 1e-6) or whose genetic position was not known. The final marker set that passed quality control included 45363 SNPs from 39 samples. The positions of the SNPs were based on the UMD3.1 assembly (ftp://ftp.cbcb.umd.edu/pub/data/assembly/Bos_taurus/Bos_taurus_UMD_3.1/).

The SNPs were sorted by association P-values and their output was saved in tab-delimited and txt format. The markers with the most significant association were extracted (at least 10 SNPs for each trait), presented in the results tables and further analyzed. Genome-wide significance was set to the standard GWAS threshold of $p < 5 \times 10^{-8}$ and suggestive significance was set to $p < 1 \times 10^{-5}$. An FDR cut-off value of < 0.1 was applied for the type I error. Due to the small sample size, strict criteria applying the Bonferroni correction were not applied in the analysis and discussion of the results but were only used to highlight which results met the criteria. Various databases were used to identify candidate genes in the vicinity of SNPs associated with the traits studied. Databases such as Ensembl (www.ensembl.org) and NCBI (www.ncbi.nlm.nih.gov) were used to identify candidate genes in the vicinity of SNPs associated with the studied traits. Genes at the position of SNP or within a distance of 0.3 Mb were selected for the literature search. Descriptive statistics and correlation matrices were generated with the statistics package Statsoft Statistica 12.0 (Statsoft, 2013).

3 Results

Table 1 shows the summary of meat quality traits with emphasis on mean, minimum and maximum value and standard deviation (SD). The standard deviation is a measure of the amount of variation or dispersion in a set of values and indicates for the analyzed meat quality traits that the data points are close to the mean (protein content and pH) or that there is a moderate level of variability (tenderness).

Table 2 shows the summary of the fatty acid profile in meat. Certain fatty acids (e.g. 18:3 n-3, 16:1 n-7) were present in some samples in extremely low amounts, so the method used in this study could not detect them. For this reason, descriptive statistics summarise values that could be determined in samples. According to the results of this study, the most common fatty acids are the SFAs and the least common are the PUFAs. Of the induvial fatty acids, oleic fatty acid has the highest average content. Table 3 shows the summary of the correlations between the meat quality traits and the fatty acid profile. The protein content (%) has a negative correlation with the pH value and a positive correlation with the PUFAs (%). SFAs had a negative correlation with the MUFAs and PUFAs. Table 4 shows the summary of the correlations between the meat quality traits and four individual fatty acids. The pH value correlates negatively with myristic and palmitic fatty acid, but positively with stearic fatty acid. The protein content has a positive correlation with the linoleic fatty acid. Stearic fatty acid has a negative correlation with palmitic fatty acid and oleic fatty acid.

GWA study was conducted on the five meat quality traits (protein content, pH, water holding capacity, tenderness and muscle fiber diameter) and the fatty acid profile of Busha meat from young bulls from Dimitrovgrad, Serbia. SNPs that reached genome-wide significance are listed in Table 5. Thirteen SNPs were associated with muscle fiber diameter and one with pH. All of these SNPs had an FDR < 0.1. The proteins coding gene positioned near the detected SNPs are also listed in Table 5. The genomic inflation coefficients were $\lambda = 1.071$ for muscle fiber diameter and $\lambda = 1.076$ for pH. None of the SNPs reached the genome-wide significance threshold for association with FA.

The number of SNPs with suggestive significance and SNPs with significance cut-off values of $p < 0.001$ and $p < 0.01$ for each trait is presented in Table 6.

For significantly correlated traits, a search for common SNPs was performed among those that had a significance cut-off $p < 0.001$. No such SNPs were identified for protein content and pH or protein content and PUFA. SNPs associated with any of the previously mentioned traits could only be identified at lower inclusion criteria $p < 0.01$ (data not shown). Only a

Tab. 1. Summary of meat quality traits
Zusammenfassung der Fleischqualitätsmerkmale

Meat traits	N	Mean	Minimum	Maximum	SD
Protein content (%)	18	21.64	20.09	22.92	0.72
pH	39	5.69	5.44	6.64	0.21
WHC (cm^2)	39	12.11	9.5	13.96	1.22
Tenderness (kg)	21	8.20	5.02	11.5	1.33
Muscle fiber diameter (μm)	23	32.59	27.88	38.09	2.39

N – number of observations per trait; SD – standard deviation; WHC – Water-holding capacity.

Tab. 2. Summary of fatty acids profile
Zusammenfassung des Fettsäureprofils

Fatty acids	N	Mean	Minimum	Maximum	SD
14:00	14	5.52	0.63	16.25	3.79
16:00	37	35.60	25.56	54.39	6.49
16:1 n-7	7	4.73	0.66	13.55	4.57
18:00	37	20.03	6.16	29.72	5.46
18:1c n-9	37	36.13	25.71	54.73	7.18
18:2c n-6	24	7.99	0.52	16.45	4.32
18:3 n-3	2	1.85	0.92	2.77	1.31
SFA	37	57.70	45.27	72.54	7.03
MUFA	37	37.02	25.71	54.73	7.29
PUFA	24	8.14	0.52	16.45	4.36
n-6/n-3 ratio	2	7.44	2.43	12.45	7.09

N – number of observations per trait; SD – standard deviation; 14:00 – myristic fatty acid; 16:00 – palmitic fatty acid; 16:1 n-7 – palmitoleic fatty acid; 18:00 – stearic fatty acid; 18:1c n-9 – oleic fatty acid; 18:2c n-6 – linoleic fatty acid; 18:3 n-3 – α-linolenic fatty acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 – omega-6 fatty acids; n-3 – omega-3 fatty acids; n-6/n-3 ratio – for the ratio, meat samples were used in which both n-6 and n-3 fatty acids could be determined according to the method used.

Tab. 3. Correlations of meat quality traits and fatty acids content
Korrelationen von Fleischqualitätsmerkmalen und Fettsäuregehalt

Traits	Protein content (%)	pH	WHC (cm^2)	Tenderness (kg)	Diameter (µm)	SFA	MUFA	PUFA
Protein content (%)	1	**-0.64***	–0.20	–	0.13	–0.04	–0.49	**0.58***
pH	-	1	0.05	0.04	–0.03	–0.04	0.26	–0.25
WHC (cm^2)	–	–	1	–0.002	0.02	0.18	–0.23	0.03
Tenderness(kg)	–	–	–	1	–	–0.15	–0.02	0.07
Diameter (µm)	–	–	–	–	1	–0.06	–0.30	0.48
SFA	–	–	–	–	–	1	**-0.68***	**-0.52***
MUFA	–	–	–	–	–	–	1	–0.27
PUFA	–	–	–	–	–	–	–	1

Marked correlations* are significant at $p < 0.05$; WHC – Water holding capacity; Diameter – Muscle fiber diameter (µm); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids.

Tab. 4. Correlations of meat quality traits with fatty acids content
Korrelationen von Fleischqualitätsmerkmalen mit dem Gehalt an Fettsäuren

Traits	14:00	16:00	18:00	18:1c n-9	18:2c n-6
Protein content (%)	0.78	–0.17	–0.11	–0.11	**0.58***
pH	**-0.57***	**-0.35***	**0.34***	–0.12	–0.26
WHC (cm^2)	0.21	–0.14	0.12	–0.21	0.04
Tenderness (kg)	–0.67	–0.26	0.14	–0.02	0.07
Diameter (µm)	0.46	–0.09	–0.08	–0.31	0.47
14:00	1	0.05	–0.43	–0.12	–0.49
16:00	–	1	**-0.38***	–0.25	–0.05
18:00	–	–	1	**-0.41***	–0.20
18:1c n-9	–	–	–	1	0.04
18:2c n-6	–	–	–	–	1

Marked correlations (*) are significant at p <.05000; WHC – Water-holding capacity; Diameter – Muscle fiber diameter (µm); 14:00 – myristic fatty acid; 16:00 – palmitic fatty acid; 18:00 – stearic fatty acid; 18:1c n-9 – oleic fatty acid; 18:2c n-6 – linoleic fatty acid.

few SNPs were common to both FA 16:00 and FA 18:00: BTA-112619-no-rs ($p_{16:00}$ = 0.00054 and $p_{18:00}$ = 0.00041), BTB-01174768 ($p_{16:00}$ = 0.0012 and $p_{18:00}$ = 0.00063) and BTB-01951920 ($p_{16:00}$ = 0.00034 and $p_{18:00}$ = 0.00024), which is also significantly associated with pH (ph = 0.0010). ARS-BFGL-NGS-118200 was significant for FA 16:00 and borderline significant for FA 18:00 ($p_{16:00}$ = 0.00089 and $p_{18:00}$ = 0.0011). FA 18:00 and 18:01 had Hapmap48202-BTA-118947 ($p_{18:00}$ = 0.0011 and $p_{18:01}$ = 0.00050) and ARS-BFGL-NGS-40496 ($p_{18:00}$ = 0.00107 and $p_{18:01}$ = 0.00055) as a common SNP.

4 Discussion

This study represents one of the first analyses of meat quality traits in Busha cattle in Serbia. Meat production in extensive production systems is the focus of most Busha breeders, so understanding the meat quality traits and the correlation between them is of great importance.

In this study, the average protein content was 21.64%, which corresponds to the results of Cheng et al. (2020) with 21.74% in Hanwoo breed beef loin and the results of Petričević (2018) with 22.31% in M. longissimus dorsi of the Simmental breed. The pH value is an important parameter for meat quality, and values between 5.48 and 5.79 indicate that there are no stress-related meat defects such as dry or dark meat (López-Pedrouso et al., 2020). Xie et al. (2012) analyzed the meat quality of three local cattle breeds in China and reported pH values between 5.61 and 5.73. These pH values are consistent with the results of this study, which show that the average pH value of Busha meat was 5.69. Some studies indicate a significant influence of production systems on the variation of meat pH in bulls of local breeds (Guerrero et al., 2013; Humada et al., 2014), while other authors found no such influence (López-Pedrouso et al., 2020). The average water-holding capacity in this study was 12.11 cm^2, which is higher compared to the results of Petričević (2018) with 11.04 cm^2 in M. longissimus dorsi of the Simmental breed. The water-holding capacity of the meat can

Tab. 5. Genome-wide significant associations of SNPs with meat quality traits and fatty acids composition
Genomweite signifikante Assoziationen von SNPs mit Fleischqualitätsmerkmalen und der Fettsäurezusammensetzung

SNP	p	FDR_BH	FDR_BY	CHR	Position	Adjacent gene#
ARS-BFGL-NGS-117761	0.0000000001795	0.0000006507	0.000007326	1	72080335	***APOD***
ARS-BFGL-NGS-110100	0.0000000001795	0.0000006507	0.000007326	16	37982355	***NTMT2***
BTA-96884-no-rs	0.0000000001795	0.0000006507	0.000007326	16	55261770	***ZBTB37***
Hap-map47945-BTA-41852	0.0000000001795	0.0000006507	0.000007326	17	6295259	
BTB-00669586	0.0000000001795	0.0000006507	0.000007326	17	6322271	
ARS-BFGL-NGS-55320	0.0000000001795	0.0000006507	0.000007326	20	829627	
ARS-BFGL-NGS-56305	0.0000000001795	0.0000006507	0.000007326	24	26333813	***DSC1, DSC2, DSC3***
BTB-01370348	0.0000000001795	0.0000006507	0.000007326	24	32719091	
BTB-01623856	0.0000000001795	0.0000006507	0.000007326	24	32938309	
ARS-BFGL-NGS-21254	0.0000000001795	0.0000006507	0.000007326	29	1898171	***SCL36A4***
Hap-map33716-BTA-140311	0.0000000001795	0.0000006507	0.000007326	29	2858775	***FAT3***
Hap-map42621-BTA-65545	0.0000000001795	0.0006507	0.007326	29	35481937	***NTM***
ARS-BFGL-BAC-28908	0.000000000486	0.001626	0.01831	23	16536674	***RPL7L1***
BTB-00613588*	0.000000005873	2.56e-07	0.002883	15	71342153	***API, TTC17***

SNP – Single Nucleotide Polymorphism, SNPs associated with muscle fiber diameter; * SNP associated with pH; Significance threshold set at p < 0.00000001; FDR_BH -False Discovery Rate (FDR) using the Benjamini & Hochberg procedure; FDR_BY – False Discovery Rate (FDR) using the Benjamini & Yekutieli procedure; CHR – chromosome.

be influenced by the breed (López-Pedrouso et al., 2020; Moreno-Indias et al., 2011). Petričević (2018) reported that the average meat tenderness was 10.61 kg for the Simmental breed which is higher compared to the results presented in this study. In this study, the average diameter of the muscle fibers was 32.59 μm. Zheng et al. (2018) analyzed the muscle fiber diameter in Jinjiang yellow cattle and reported the following results: highrib (31.71 μm),

Tab. 6. Number of SNPs associated with investigated traits with regard to significance
Anzahl der mit den untersuchten Merkmalen assoziierten SNPs in Bezug auf Signifikanz

Trait	FDR_BH < 0.1	p < 1x10^{-5}	p < 0.001	p < 0.01
Protein content (%)	0	0	17	281
pH	12	5	66	353
WHC (cm^2)	0	1	74	682
Tenderness (kg)	0	0	26	459
DMF (μm)	31	16	89	453
14:00	0	0	78	616
16:00	0	0	35	403
16:1 n-7	0	0	1	142
18:00	2	2	97	753
18:1c n-9	0	0	127	1114
18:2c n-6	0	0	25	323
18:3 n-3	/	/	/	/
SFA	0	0	26	385
MUFA	0	0	103	830
PUFA	0	0	29	308
n-6	0	0	25	323
n-3	/	/	/	/
n-6/n-3 ratio	/	/	/	/

FDR_BH -False Discovery Rate (FDR) using the Benjamini & Hochberg procedure; WHC – Water-holding capacity; DMF – Muscle fiber diameter (μm); 14:00 – myristic fatty acid; 16:00 – palmitic fatty acid; 16:1 n-7 – palmitoleic fatty acid; 18:00 – stearic fatty acid; 18:1c n-9 – oleic fatty acid; 18:2c n-6 – linoleic fatty acid; 18:3 n-3 – α-linolenic fatty acid; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; n-6 – omega-6 fatty acids; n-3 – omega-3 fatty acids; n-6/n-3 ratio – for the ratio, meat samples were used in which both n-6 and n-3 fatty acids could be determined according to the method used.

tenderloin (33.78 μm) and ribeye (31.63 μm). The results of this study are essentially consistent with those of other studies. The tenderness of the meat, as well as the diameter of muscle fibers and water holding capacity, can be influenced by factors such as the breed, age at slaughter, and the timing of meat sampling (before or after rigor mortis).

The results of this study show that, on average, saturated fatty acids were the most common (57.70%), followed by monounsaturated fatty acids (37.02%) and polyunsaturated fatty acids (8.14%). A high consumption of SFA from 12:00 (lauric acid) to 18:00 (stearic acid) can increase the risk of coronary heart disease (Zong et al., 2016). In this study, the SFAs with the highest content in total fatty acids were palmitic fatty acid, stearic fatty acid and myristic fatty acid. Xie et al. (2012) also reported that these three SFA fatty acids were the most abundant in five cattle breeds. Myristic acid (14:00) and palmitic acid (16:00) affect low-density lipoprotein (LDL), which is referred to as "bad cholesterol", but have little effect on the ratio of total cholesterol to HDL (Micha and Mozaffarian, 2010). Stearic acid is the only SFA unlikely to increase cardiovascular disease risk (Nogoy et al., 2022). Oleic acid was the most

abundant fatty acid in meat in this study which is consistent with the findings of Xie et al. (2012) and Moreno-Indias et al. (2011). Hammad et al. (2016) reported the positive role of MUFAs in human cardiovascular health. According to results presented in this study, linoleic fatty acid is the most abundant PUFA in meat. This result is consistent with other studies (Xie et al., 2012; Zheng et al., 2018). Linoleic acid n-6 and α-linolenic acid n-3 are essential fatty acids and the most important PUFAs in the human diet (Nogoy et al., 2022).

Understanding the correlation between variables allows for better prediction. The pH value is one of the most important parameters influencing the meat WHC (Bendall and Swatland, 1988; Watanabe et al., 2018). Farouk et al. (2012) found that WHC correlates positively with the pH of M. semimembranosus in cattle. The results of this study show that the correlation between pH and WHC was positive but not significant. Du et al. (2021) also found a positive but non-significant correlation between pH and WHC in Chinese Simmental beef. Jankowiak et al. (2021) reported that pH values were highly significantly and positively correlated with tenderness and water content and negative correlations were recorded with WHC and protein content ($P < 0.01$). In this study, pH values were also negatively correlated with protein content ($P < 0.05$), indicating that meat samples with a higher pH value had a lower protein content which is consistent with the results of Jankowiak et al. (2021). Kim et al. (2016) found a negative correlation between pH and protein content, indicating that the higher values of protein content increase the rate of decline in pH.

Negative correlations (Jankowiak et al., 2021) and positive correlations (Farouk et al., 2012) between WHC and pH values were probably due to different methods for determining the WHC of meat. The relationship between pH and WHC was not consistent across studies, including this one. Although there was a significant correlation between pH and individual SFAs in this study, it is important to note that the correlation between these two parameters is complex and probably not straightforward and has not been reported by other authors. The correlation between WHC and meat tenderness (kg) was negative but not significant in this study, which is consistent with the results of Rostamani et al. (2021) and Du et al. (2021). In this study protein content was significantly correlated with PUFAs, indicating that the increase in meat protein content leads to an increase in PUFAs. While protein is a consistent component of meat, fat composition, particularly PUFAs, can be very variable, so further research is needed to confirm the correlation between these meat quality traits.

In this study, a significant negative correlation of SFA with MUFA and PUFA was found, which was the expected result. Hwang and Joo (2017) found that SFA and PUFA had a positive and significant correlation with meat tenderness (shear force), while MUFA had a significant negative correlation with meat tenderness (shear force). In this study, the correlations between meat tenderness and SFA and MUFA were negative and for meat tenderness and PUFA were positive, although not significant. Garmyn et al. (2011) found that stearic acid and PUFA were positively correlated ($p < 0.05$) with tenderness (Warner-Bratzler shear force), while MUFA was negatively associated ($p < 0.05$) with the same trait.

There is a lack of genetic data on the Serbian Busha cattle population. The autochthonous cattle breeds have always been part of a specific environment to which they were adapted. The current study is the first GWAS on meat quality traits in this domestic animal. By applying SNP analysis and identifying animals with favorable traits, it is possible to select animals that have specific traits as part of programs to improve production traits.

Fourteen SNPs of genome-wide significance have been, 13 of which are associated with muscle fiber diameter and one with meat pH. For these SNPs, the FDR was significantly higher than the cut-off value of 0.1. Only a few of these 13 SNPs are located near proteins with scientifically proven effects on muscle and muscle fibers. ARS-BFGL-NGS-117761 is located near the gene for apolipoprotein D (APOD). ApoD regulates tissue homeostasis by keeping lipid peroxidation at a low level (Ganfornina et al., 2008). Differential expression analysis of mRNA in chicken gastrocnemius muscle was performed and it was shown that

ApoD protects against muscle atrophy under dissuasive stress. In addition to muscle atrophy, muscle fiber diameter was also reduced (Mo et al., 2022). NTMT2, adjacent to ARS-BFGL-NGS-110100, is increasingly expressed during osteogenic and myogenic differentiation (Tooley et al., 2021). The transcript level of ZBTB37 adjacent to BTA-96884-no-rs is significantly increased during the acute phase of exercise in the soleus muscle of diabetic KK-Ay mice with peripheral arterial disease (Nagase et al., 2017). Despite the small number of samples analyzed, it can be suggested that the SNPs ARS-BFGL-NGS-117761, ARS-BFGL-NGS-110100 and BTA-96884-no-rs and adjacent genes should be investigated in further studies in relation to muscle traits.

The fatty acid composition of beef influences the flavor, aroma and sensory properties of the meat (Venkata Reddy et al., 2015). Fatty acid composition has been shown to vary between breeds and is influenced by nutrition (Venkata Reddy et al., 2015; Vahmani et al., 2015). The use of genomic markers and regions associated with the presence of fatty acids in beef can facilitate the selection process to modify the ratio of saturated to monounsaturated or polyunsaturated fatty acids, thus contributing to better results.

Several SNPs have been identified that are likely to be associated with two or more traits under analysis. Hapmap48202-BTA-118947 (associated with FA 18:00 p = 0.00095 and FA 18:01 p = 0.00042) is associated with meat quality traits in a GWAS study of three French beef cattle breeds, with SNPs considered significant at a 5 × 10–4 threshold (Allais et al., 2014). It has also been associated with lactation persistence in Canadian Holstein cattle (Do et al., 2017). BTB-01951920 (associated with FA 16:00 p = 0.00056 and FA 18:00 p = 0.00035) was previously identified in a GWA study as a SNP with suggestive significance for mineral (Cu) abundance in the milk of Vrindavani crossbred cattle (Singh et al., 2022). It is located in the intronic region of the MRPS30 gene, which plays a role in apoptosis (Appuhamy et al., 2009). The identified SNPs that were associated with two different traits, BTA-112619-no-rs,BTB-01174768 and ARS-BFGL-NGS-40496 have not been investigated in animal studies and are not located near the identified genes while the Hapmap48202-BTA-118947 was identified as a marker for lactation persistency in Canadian Holstein cattle (Do et al., 2017). Hapmap41060-BTA-68516 was associated with FA 18:2 (p = 0.000021) and PUFA (p = 0.000044). It is located near the gene for hepatic angiopoietin-like protein 3 (*ANGPTL3*), which is predominantly expressed in the liver and plays an important role in regulating circulating triglyceride and lipoprotein fractions by inhibiting lipoprotein lipase activity. ANGPTL3 is associated with changes in lipid metabolism during the fattening period and may influence blood lipid metabolites (Shikida et al., 2023). Some of the common SNPs associated with tenderness are located in μ-calpain and calpastatin in Bos Taurus populations. No association was found for these SNPs.

Leal-Gutiérrez et al. (2019) reported the lack of association in their study and explained this with the genomic influence of Bos Indicus on the breed. Busha is not a highly selected breed and it could be that the association is not as strong as in other breeds.

The main limitation of this study is the limited number of samples. A larger sample would provide more reliable data for GWAS analysis. Yet, as such study was performed for the first time in Busha cattle it provides valuable novel insights. Appropriate measures have been taken to mitigate and highlight potential false positives. At this stage, however, the results should be regarded as preliminary and suggest further studies with larger samples.

5 Conclusion

This is the first report on GWAS on some meat quality traits in Serbian Busha cattle. Despite the limitations of this study, several SNPs and genes that could potentially influence meat quality and fatty acid profile were reported in Busha cattle. The discovery of these genes

provides valuable perspectives on Busha genetics that offer potential applications for improving meat production traits in this autochthonous cattle breed. Several protein traits and fatty acids could be correlated and these characteristics should be taken into account when crossing breeds.

Acknowledgment

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, on the basis of the Agreements on the realization and financing of scientific research work of SRO No. 451–03–66/2024–03/200022 and No. 451–03–66/2024–03/200017.

Literatur

Allais, S., H. Levéziel, J.F. Hocquette, S. Rousset, C. Denoyelle, L. Journaux and G. Renand (2014): Fine mapping of quantitative trait loci underlying sensory meat quality traits in three French beef cattle breeds. J Anim Sci. **92,** 4329–41.

Appuhamy, J.A., B. Cassell and J. Cole (2009): Phenotypic and genetic relationships of common health disorders with milk and fat yield persistencies from producer-recorded health data and test-day yields. J. Dairy Sci. **92**, 1785–1795.

Bendall, J.R. and H.J. Swatland (1988): A review of the relationships of pH with physical aspects of pork quality. Meat Science **24**, 85–126.

Bolormaa, S., J.E. Pryce, K.E. Kemper, B.J. Hayes, Y. Zhang, B. Tier, W. Barendse, A. Reverter and M.E. Goddard (2013): Detection of quantitative trait loci in Bos indicus and Bos taurus cattle using genome-wide association studies. Genet Sel Evol. **45**, 43.

Briggs, M.A., K.S. Petersen and P.M. Kris-Etherton (2017): Saturated Fatty Acids and Cardiovascular Disease: Replacements for saturated fat to reduce cardiovascular risk. Healthcare (Basel, Switzerland), 5 (2), 1 – 29.

Cheng, H., S. Song, E.Y. Jung, J.Y. Jeong, S.T. Joo and G.D. Kim (2020): Comparison of beef quality influenced by freeze-thawing among different beef cuts having different muscle fiber characteristics. Meat Science **169**, 1 – 7.

Do, D.N., N. Bissonnette, P. Lacasse, F. Miglior, M. Sargolzaei, X. Zhao and E.M. Ibeagha-Awemu (2017): Genome-wide association analysis and pathways enrichment for lactation persistency in Canadian Holstein cattle. J Dairy Sci. **100**, 1955–1970.

Du, L., T. Chang, B. An, M. Liang, X. Duan, W. Cai, B. Zhu, X. Gao, Y. Chen, L. Xu, L. Zhang, J. Li and H. Gao (2021): Transcriptome profiling analysis of muscle tissue reveals potential candidate genes affecting water holding capacity in Chinese Simmental beef cattle. Sci Rep. **11**, 11897.

Farouk, M.M., N.M. Mustafa, G. Wu and G. Krsinic (2012): The„sponge effect" hypothesis: An alternative explanation of the improvement in the waterholding capacity of meat with ageing. Meat Science **90**, 670–677.

Folch, J., M. Lees and G.H. Sloane-Stanley (1957): A simple method for the isolation and purification of total lipides from animal tissues. J. Biol. Chem. **226**, 497–507.

Ganfornina, M.D., S. Do Carmo, J.M. Lora, S. Torres-Schumann, M. Vogel, M. Allhorn, C. González, M.J. Bastiani, E. Rassart and D. Sanchez (2008): Apolipoprotein D is involved in the mechanisms regulating protection from oxidative stress. Aging Cell **7**, 506–515.

Garmyn, A.J., G.G. Hilton, R.G. Mateescu, J.B. Morgan, J.M. Reecy, R.G. Tait, D.C. Beitz, Q. Duan, J.P. Schoonmaker, M.S. Mayes, M.E. Drewnoski, Q. Liu and D.L.

VanOverbeke (2011): Estimation of relationships between mineral concentration and fatty acid composition of longissimus muscle and beef palatability traits, J. Anim. Sci. **89**, 2849–2858.

Geletu, U.S., M.A. Usmael, Y. Mummed and A.M. Ibrahim (2021): Quality of Cattle Meat and Its Compositional Constituents. Veterinary medicine international 7340495, 1–9.

Grau, R., R. Hamm und A. Baumann (1953): Über das wasserbindungsvermögen des toten Säugetiermuskels. I. Mitteilung. Der Einfluß des pH Wertes auf die Wasserbindung von zerkleinertem Rindermuskel. Biochem. Z. **325**, 1–11.

Guerrero, A., C. Sañudo, P. Albertí, G. Ripoll, M.M. Campo, J.L. Olleta, B. Panea, S. Khliji and P. Santolaria (2013): Effect of production system before the finishing period on carcass, meat and fat qualities of beef. Animal: Intern. J. Anim. Biosci. **7**, 2063–2072.

Hammad, S., S. Pu and P.J. Jones (2016): Current Evidence Supporting the Link Between Dietary Fatty Acids and Cardiovascular Disease. Lipids **51**, 507–517.

Haynes, G.D. and E.K. Latch (2012): Identification of novel single nucleotide polymorphisms (SNPs) in Deer (Odocoileus spp.) using the BovineSNP50 BeadChip. *PLOS One*. 2012; 7(5): 11.

Humada, M.J., C. Sañudo and E. Serrano (2014): Chemical composition, vitamin E content, lipid oxidation, colour and cooking losses in meat from Tudanca bulls finished on semi-extensive or intensive systems and slaughtered at 12 or 14 months. Meat science **96,** Pt A, 908–915.

Hwang, Y.H. and S.T. Joo (2017): Fatty acid profiles, meat quality, and sensory palatability of grain-fed and grass-fed beef from Hanwoo, American, and Australian crossbred cattle. Korean J. Food Sci. Anim. Resour. **37**, 153–161.

Jankowiak, H., A. Cebulska and M. Bocian (2021): The relationship between acidification (pH) and meat quality traits of polish white breed pigs. European Food Research and Technology **247**, 2813–2820.

Kim, T.W., C.W. Kim, S.G. Kwon, J.H. Hwang, D.H. Park, D.G. Kang, J. Ha, M.R. Yang, S.W. Kim and I.-S. Kim (2016): PH as analytical indicator for managing pork meat quality. Sains Malaysiana **45**, 1097–1103.

Leal-Gutiérrez, J.D., M.A. Elzo, D.D. Johnson, H. Hamblen and R.G. Mateescu (2019): Genome wide association and gene enrichment analysis reveal membrane anchoring and structural proteins associated with meat quality in beef. BMC Genomics **20**, 151.

Listrat, A., M. Gagaoua, D. Andueza, D. Gruffat, J. Normand, G. Mairessec, B. Picard and J.F. Hocquettea (2020): What are the drivers of beef sensory quality using metadata of intramuscular connective tissue, fatty acids and muscle fiber characteristics? Livest. Sci. **240**, 1 – 14.

López-Pedrouso, M., R. Rodríguez-Vázquez, L. Purriños, M. Oliván, S. García-Torres, M.Á. Sentandreu, J.M. Lorenzo, C. Zapata and D. Franco (2020): Sensory and physicochemical analysis of meat from bovine breeds in different livestock production systems, pre-slaughter handling conditions and ageing time. Foods **9**, 176.

Micha, R. and D. Mozaffarian (2010): Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. Lipids **45**, 893–905.

Mo, J., Z. Wang, Q. Liu, Z. Li and Q. Nie (2022): Construction and analysis of disuse atrophy model of the gastrocnemius muscle in chicken. Int. J. Mol. Sci. **23**, 6892.

Moreno-Indias, I., L.E. Hernández-Castellano, A. Morales-delanuez, N. Castro, J. Capote, V. Mendoza-Grimón, M.A. Rivero and A. Argüello (2011): Differences on meat quality of local cattle breed from outermost EU zone vs. commercial. J. Appl. Anim. Res. **39**, 328–333.

Nagase. H., S. Yao and S. Ikeda (2017): Acute and chronic effects of exercise on mRNA expression in the skeletal muscle of two mouse models of peripheral artery disease. PLoS One. **12**, e0182456.

Nogoy, K.M.C., B. Sun, S.Shin, Y. Lee, X. Zi Li, S.H. Choi and S. Park (2022): Fatty Acid Composition of Grain- and Grass-Fed Beef and Their Nutritional Value and Health Implication. Food Sci. Anim. Res. **42**, 18–33.

Petričević, M. (2018): Quality of bovine carcasses and meat in the function of using flax seeds in food. Diss. Univ. Belgrade (SRB).

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M.A.Ferreira, D. Bender, J. Maller, P. Sklar, P.I. de Bakker, M.J. Daly and P.C. Sham (2007): PLINK: a tool set for whole-genome association and population-based linkage analyses. Am. J. Hum. Genet. **81**, 559–575.

Rostamani, M., H. Baghaei and M. Bolandi (2021): Prediction of top round beef meat tenderness as a function of marinating time based on commonly evaluated parameters and regression equations. Food Sci Nutr. **9**, 5006–5015.

Shikida, R., M. Kim, M. Futohashi, K. Nishihara, H. Lee, Y. Suzuki, Y. Baek, T. Masaki, K. Ikuta, E. Iwamoto, Y. Uemoto, S. Haga, F. Terada and S. Roh (2023): Physiological roles and regulation of hepatic angiopoietin-like protein 3 in Japanese Black cattle (Bos taurus) during the fattening period. J Anim Sci. **101**, skad198.

Singh, A., A. Kumar, C. Gondro, A.K. Pandey, T. Dutt and B.P. Mishra (2022): Genome wide scan to identify potential genomic regions associated with milk protein and minerals in Vrindavani cattle. Front Vet Sci. **9**, 760364.

SRPS EN ISO (5509) 2007: Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids.

SRPS ISO 937 (1992): Određivanje sadržaja belančevina/Determination of protein content.

Statsoft (2013): Statistica for Windows, release 12.0, Inc., Tulsa, OK, USA.

Tooley, J.G., J.P. Catlin and C.E.S. Tooley (2021): CREB-mediated transcriptional activation of NRMT1 drives muscle differentiation. Transcription **12**, 72–88.

Vahmani, P., C. Mapiye, N. Prieto, D.C. Rolland, T.A. McAllister, J.L. Aalhus and M.E.R. Dugan (2015): The scope for manipulating the polyunsaturated fatty acid content of beef: A review. J. Anim. Sci. Biotechnol. **6**, 29.

Venkata Reddy, B., A.S. Sivakumar, D.W. Jeong, Y.B. Woo, S.J. Park, S.Y. Lee, J.-Y. Byun, C.H. Kim, S.H. Cho and I. Hwang (2015): Beef quality traits of heifer in comparison with steer, bull and cow at various feeding environments. J. Anim. Sci. **86**, 1–16.

Visscher, P.M., M.A. Brown, M.I. McCarthy and J. Yang (2012): Five years of GWAS discovery. Am. J. Hum. Genet. **90**, 7–24.

Volodkevich, N.N. (1938): Apparatus for measurement of chewing resistance or tenderness of foodstuffs. Food Res. **3**, 221–225.

Watanabe, G., M. Motoyama, I. Nakajima and K. Sasaki (2018): Relationship between water-holding capacity and intramuscular fat content in Japanese commercial pork loin. Asian-Australasian J. Anim. Sci. **31**, 914–918.

Xie, X., Q. Meng, Z. Cui and L. Ren (2012): Effect of cattle breed on meat quality, muscle fiber characteristics, lipid oxidation and fatty acids in China. Asian-Australasian J. Anim. Sci. **25**, 824–831.

Zalewska, M., K. Puppel and T. Sakowski (2021): Associations between gene polymorphisms and selected meat traits in cattle – A review. Anim Biosci. **34**, 1425–1438.

Zheng, Y., S. Wang and P. Yan (2018): The meat quality, muscle fiber characteristics and fatty acid profile in Jinjiang and F1 Simmental×Jinjiang yellow cattle. Asian-Australasian J. Anim. Sci. **31**, 301–308.

Zong, G., Y. Li, A.J. Wanders, M. Alssema, P.L. Zock, W.C. Willett, F.B. Hu and Q. Sun (2016): Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. BMJ (Clinical research ed.) **355**, i5796, 1 – 11.