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**ORIGINAL PAPER**

# HERBAL EXTRACTS IN THE DIET OF HOLSTEIN-FRIESIAN BULLS CHANGE THE NUTRITIONAL VALUE OF *LONGISSIMUS LUMBORUM* AND *SEMIMEMBRANOSUS* MUSCLES

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**Abstract**

The effects of herbal extracts included in diets for Holstein-Friesian (HF) bulls on the fatty acid profile, vitamin and mineral content of *Longissimus lumborum* (LL) and *Semimembranosus* (SM) muscles were evaluated to determine their potential beneficial influence on meat quality. The biochemical profile of bovine serum was determined. At six months of age, twenty four bulls were randomly assigned to one of three dietary treatments: diet C (control), diet O (supplemented with the herbal preparation O), and diet OS (supplemented with herbal preparations O and S). At the end of the fattening period (mth 20), the animals were slaughtered, carcass traits were evaluated, and samples of LL and SM muscles were collected to determine the content of functional fatty acids, vitamins and minerals. Dietary supplementation with herbal extracts O and OS increased the content of vitamin E and minerals (Na, Fe, Zn) in the analyzed muscles. The fat content of the muscles was affected by dietary treatments ($P<0.01$). The SM muscle was characterized by a lower content of fat with a more desirable fatty acid profile, compared with the LL muscle. Dietary supplementation with composite herbal preparations improves the nutritional value of meat from HF bulls.

**Keywords:** beef, biochemical blood parameters, fatty acids composition, herbal extracts, minerals, nutritional value

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## INTRODUCTION

Medicinal plants deliver health benefits to cattle and improve the quality of food products consumed by humans, beef and milk (Monteschio et al. 2019, Rivaroli et al. 2016), which indirectly affect human health. More desirable effects are observed following long-term repeated administration of herbal feed additives. Properly selected herbal blends, adjusted to animal species, age and production profile, deliver greater benefits than single herbs (Czech et al. 2009). However, potential antagonistic interactions and pharmacological synergism between the active ingredients of herbs should be taken into account. Herbs and herbal blends can affect animals by improving the gastrointestinal function and exerting anti-inflammatory, antioxidant and antibacterial effects. Healthy liver function is an important economic consideration in beef cattle production because liver disorders may decrease animal performance and carcass yield. These adverse effects cannot be alleviated by antibiotic growth promoters, which has prompted the search for natural alternatives such as herbal extracts with specific properties. For instance, the herbs used in this study (including *Curcuma longa*, *Zingiber officinale* and *Alium sativum*) increase the production of bile and bile acids in the liver, and digestive enzymes such as amylase, lipase and trypsin in the pancreas (Frankič et al. 2009). Essential oils and herbal blends have shown potential to modulate lipid metabolism in the rumen, increase feed efficiency and live weight gain, thus affecting the fatty acid composition of meat and milk (Zawadzki et al. 2017, Monteschio et al. 2019). Plants rich in bitter compounds and essential oils, such as peppermint, stimulate the salivary glands to produce saliva and increase gastric acid secretion (Studzińska-Sroka et al. 2018). Herbal extracts may also influence various physiological functions. Some of the biologically active compounds found in herbs can improve the sensory and nutritional attributes of animal products, important for consumers. This is due to the fact that herbs and spices can protect feed against oxidative deterioration during storage (Bhatt 2015). The efficacy of some herb species (rosemary, oregano, peppermint, thyme, cinnamon, garlic) in animal nutrition has been relatively well researched, whereas other species have not been extensively used.

The research hypothesis postulates that the addition of two multi-component herbal preparations to the diets of beef bulls affects the composition of intramuscular fat (IMF), and the content of vitamins, macronutrients and micronutrients in beef, thus improving its nutritional profile. Supplementation improves the function of the gastrointestinal tract, which is reflected in changes in the biochemical profile of bovine serum. Bovine meat is not a homogeneous product; it includes different types of skeletal muscles and internal organs, and the concentrations of essential trace elements in muscle tissue depend on the metabolism of individual muscles (Meyer, Harley 2004, Czerwonka, Szterk 2015). Therefore, two muscles – *Longissimus lumborum*

(LL) and *Semimembranosus* (SM) – located in different parts of the beef carcass and characterized by distinctly different fiber types and sensory properties – were analyzed in the present experiment.

## MATERIALS AND METHODS

### Animal ethics

The experimental protocol was approved by the Ethics Committee of the University of Warmia and Mazury (Decision No. 121/2010). Slaughter and post-slaughter processing were carried out in accordance with Council Regulation (EC) No. 1099/2009 of 24 September 2009 on the protection of animals at the time of slaughter. During the experiment, the animals remained under veterinary care and were regularly visited by an animal care specialist.

### Animals and feeding

The experiment was performed on 24 young Polish HF bulls. The bulls were reared in a conventional production system, and were fed milk replacer, hay and concentrate. From six months of age, the animals were fattened semi-intensively. They had ad libitum access to a total mixed ration (TMR) composed of maize silage (used also during the feeding trial) and 2 kg of concentrate. At the age of 15 months, when the bulls reached body weight (BW) of 430 kg ± SD, they were divided by the analogue method into three groups of eight individuals each, and were housed in three separate pens on deep bedding. Feed intake was recorded individually with the use of the Roughage Intake Control System (Insentec BV, Marknesse, the Netherlands). In each dietary treatment group, the animals were fed ad libitum a TMR (Table 1).

All feed samples, collected once a week, were analyzed for the content of basic nutrients – with standard methods (AOAC, 2005). The content of neutral detergent fiber (NDF), assayed with heat-stable amylase and expressed exclusive of residual ash, and that of acid detergent fiber (ADF), expressed exclusive of residual ash, were determined by the method proposed by Van Soest et al. (1991) using an ANKOM220 fiber analyzer (ANKOM Technology Corp., Macedon, NY, USA). The nutritional value of diets, including net energy (meat production units, UFV), protein digested in the small intestine depending on rumen-degraded protein (PDIN) and protein digested in the small intestine depending on rumen-fermented organic matter (PDIE), were determined using the WINWAR program (Kowalski, Kański 1993). A five-month fattening period was preceded by a two-week adaptation period. During fattening, TMR was administered from a self-propelled feed cart (Seko, Curtarolo, Italy), and it was delivered to feeding stations twice daily (at 08:00 a.m. and 04:00 p.m.). Control group (C) bulls were fed exclusively

Table 1

Chemical composition and nutritional value

| Specification | Control | O | OS |
|---|---|---|---|
| TMR[1] | ad libitum | ad libitum | ad libitum |
| herbal preparations O (g)/animal/day | - | 20.00 | 20.00 |
| herbal preparations S (g)/animal/day | - | - | 40.00 (last month of fattening) |
| Chemical composition and nutritional value of TMR | | | |
| Dry matter (g) | 472.6 | 473.3 | 474.8 |
| on DM basis (g $kg^{-1}$) | | | |
| Organic matter | 938.3 | 938.3 | 938.4 |
| Total protein | 119.7 | 119.8 | 119.9 |
| Crude fat | 24.60 | 24.6 | 24.60 |
| Crude fiber | 163.7 | 163.9 | 164.2 |
| NDF | 359.4 | 360.1 | 360.9 |
| ADF | 178.5 | 178.9 | 179.7 |
| NFC | 446.3 | 445.9 | 445.1 |
| UFV | 0.920 | 0.900 | 0.900 |
| PDIN | 77.20 | 77.30 | 77.60 |
| PDIE | 90.90 | 91.00 | 91.20 |

TMR, total mixed ration, composed of maize silage and concentrate at a ratio of 65:35; concentrate composition: rapeseed meal – 15.0%, triticale meal – 82.5%, mineral-vitamin premix – 2.5% (per kg: 235 g Ca, 79 g Na, 48 g P, 28 g Mg, 500 g Fe, 2000 mg Mn, 375 mg Cu, 3750 mg Zn, 50 mg J, 12.5 mg Co, 12.5 mg Se, vitamin A – 250.000 IU, vitamin D3 – 50.000 IU, vitamin E – 1000 mg, dl-alpha-tocopherol – 909.1 mg, Cargill Poland Ltd., Warsaw, Poland); DM – dry matter; NDF – neutral detergent fiber; ADF – acid detergent fiber; NFC – non-fiber carbohydrate; UFV – feed unit for meat production; PDIN – protein digested in the small intestine when rumen-fermentable N is limiting; PDIE – protein digested in the small intestine when rumen-fermentable energy is limiting; maize silage – N-$NH_3$/N total 64,3 g $kg^{-1}$; pH 3,92; number of animals – $n$=8 per treatment.

TMR. In the experimental groups, the animals received diets supplemented with the herbal preparation O, and the herbal preparation O which was combined with the herbal preparation S in the last month of fattening – OS. These were commercial preparations the composition of which is given in Table 2. Preparation O is recommended to improve the gastrointestinal function and stimulate appetite in cattle. Preparation S has immunomodulatory, adaptogenic and anti-stress properties, and it stimulates the immune system, helping animals cope with stressors related to intensive production, which explains its inclusion in the experimental diet in the last month of fattening. The preparations were thoroughly mixed with the premix, which was then included in the concentrate together with the remaining ingredients. Fattening ended when the bulls reached minimum BW of 600 kg. The animals were transported to a meat processing plant, where they stayed

Table 2

Composition of O and OS herbal preparations

| Herbal preparation O | Herbal preparation S |
|---|---|
| Fenugreek (*Trigonella foenum-graecum*) | holy basil (*Ocimum sanctum*) |
| Fire-flame bush (*Woodfordia fruticosa*) | ashwagandha (*Withania somnifera*) |
| Green chireta (*Andrographis paniculate*) | Indian gooseberry (*Phyllanthus emblica*) |
| Indian gooseberry (*Phyllanthus emblica*) | asparagus (*Asparagus racemosus*) |
| Beleric myrobalan (*Terminalia belerica*) | licorice (*Glycyrrhiza glabra*) |
| Black myrobalan (*Terminalia chebula*) | caltrop (*Tribulus terrestris*) |
| Coriander (*Coriandrum sativum*) | Indian mango (*Mangifera indica*) |
| Garlic (*Allium sativum*) | *Shilajit* herbal extracts |
| Desert date (*Balanites roxburghii*) | |
| Turmeric (*Curcuma longa*) | |
| Ginger (*Zingiber officinale*) | |
| Marking nut tree (*Semecarpus anacardium*) herbal extracts | |
| Live yeast cultures | |

in individual boxes with access to water for h 15 to 20. Experimental units were harvested during two sessions (n=12 per session). The animals were weighed before and after slaughter with an accuracy of 0.5 kg.

**Blood sampling and analysis**

Blood for analysis was sampled from the *vena caudalis mediana*, before morning feeding, on two occasions: before treatment, after a two-week adaptation period (Experimental day 1), and after treatment, prior to slaughter (Slaughter day). The samples were placed in test tubes with potassium salt of EDTA. Following centrifugation, the blood serum was frozen at a temp. of -70°C and stored for further analyses. Selected blood parameters were determined using the BS-120 biochemistry analyzer (Mindray, USA): glucose (GLU), urea (UREA), cholesterol (CHOL), triglycerides (TG), lactate dehydrogenase (LDH-L), total protein (TP) and creatinine (CREA), as well as the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

**Vitamins and minerals**

Ninety-six hours post mortem, during carcass dressing, samples of LL and SM muscles were collected from the right half-carcass of each animal. Meat samples weighing approximately 300 g were packaged in PA/PE vacuum bags at an ambient temp. of around 4°C, under standard industrial conditions. The samples were transported in an isothermal container to the research laboratory of the Department of Cattle Breeding and Milk Evaluation at the University of Warmia and Mazury in Olsztyn. The content

of vitamins A and E was determined based on the applicable standards (PN EN 12822, 2014, PN EN 12823-1, 2014), modified for the needs of this study. Chemical compounds were separated by high-performance liquid chromatography (920-LC HPLC Analytical System, Varian, USA) on a reversed--phase Polaris C18-A column (length: 250 mm, inner diameter: 4,6 mm). Alpha-tocopherol was detected by fluorescence spectroscopy, and retinol was detected with the use of the UV-visible photodiode array detector. Data were processed using the GALAXIE Chromatography Data System. The total content of alpha-tocopherol and retinol in beef was calculated in duplicate for each sample, based on external standards (calibration curves), by determining the relationship between peak area and concentration for each analyzed substance.

To determine their mineral content, samples of LL and SM muscles were homogenized, approximately 0.5 g of the material was weighed into Teflon vessels, and 7 ml of 65% nitric acid (Merck) was added. Each sample was tested in duplicate. The vessels were sealed and the samples were mineralized in a MARS Xpress 5 microwave oven (Candela, USA). Each mineralization session involved two blank samples and two samples of certified reference material. The mineralized material was cooled and quantitatively transferred to 25 ml volumetric flasks. Minerals were analyzed with the use of an atomic absorption spectrometer (Candela, USA) equipped with light sources for each element to be determined: potassium – K, sodium – Na, magnesium – Mg, zinc – Zn, iron – Fe (PN-EN 14084, 2004; PN-EN 15505, 2009).

**Fat content and fatty acid profile**

Fat was extracted from ground meat samples of LL and SM muscles by the Soxhlet method using the B-811 extraction system (Büchi, Switzerland), with hexane as a solvent (POCH, Poland). The concentrations of selected fatty acids were determined on a CP-3800 gas chromatograph (Varian, USA) equipped with a split injector and a flame-ionization detector (FID). Data were processed using the GALAXIE Chromatography Data System. Fatty acids were identified by comparing their retention times with those of commercially available reference standards purchased from Supelco (Sigma Aldrich, USA). The fatty acids were divided into the following categories: saturated fatty acids (SFAs), unsaturated fatty acids (UFAs) including monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). The following ratios were calculated: UFA/SFA, PUFA/SFA and $n$-6/$n$-3 PUFA.

**Data analysis**

The effects of a diet (C, O and OS) and muscle type (LL and SM) on the evaluated parameters (content of vitamins and minerals in meat and fatty acids in IMF) were determined by the least squares method using the formula:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk},$$

where: $Y_{ijk}$ – the value of the analyzed parameter, $\mu$ – the population mean, $A_i$ – the effect of diet (C, O, OS), $B_j$ – the effect of muscle type (LL, SM), $(AB)_{ij}$ – the diet x muscle type interaction, and $e_{ijk}$ – the random error.

The significance of differences between mean values was estimated by the Tukey's test. The effects of a diet (C, O and OS) and the date of blood sampling (Experimental day 1, Slaughter day) on the biochemical profile of bovine serum were determined by the least squares method using the formula:

$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + e_{ijk},$$

where: $Y_{ijk}$ – the value of the analyzed parameter, $\mu$ – the population mean, $A_i$ – the effect of a diet (C, O, OS), $B_j$ – the date of blood sampling (Experimental day 1, Slaughter day), $(AB)_{ij}$ – the diet x date of blood sampling interaction, and $e_{ijk}$ – the random error.

The significance of differences between mean values was estimated by the Tukey's test. All data were analyzed statistically using Statistica version 13.3 software (StatSoft, 2019).

## RESULTS

### Carcass traits

This paper is part of a larger study investigating the effects of herbal extracts added to diets for HF bulls. The proximate composition, technological properties and sensory quality of the analyzed beef as well as lipid oxidation were described by Modzelewska-Kapituła et al. (2018). In the present experiment, HF bulls were slaughtered at the same age (20.2 to 20.8 months) and final BW (591.1 to 601.2 kg). The tested herbal extracts had no influence on hot carcass weight or dressing percentage, which could be due to the fact that their inclusion levels in animal diets were low. However, as noted by Modzelewska-Kapituła et al. (2018), the application of O treatment tended to increase the BW of bulls and carcass dressing percentage.

### Vitamins and minerals

The vitamin A content of both analyzed muscles tended to decrease in response to the applied dietary treatments (Table 3). In contrast, herbal blends O and OS had a beneficial influence on vitamin E concentration in the SM muscle. The concentrations of Fe and Zn differed between muscle types, whereas the concentrations of Na, Fe and Zn were affected by dietary treatments. Iron concentration was higher in the SM muscle than in the LL muscle. The herbal extracts contributed to a significant increase in Fe content in the LL muscle. Iron concentration was considerably lower in the

Table 3

Effects of dietary supplementation with O and OS herbal preparations and muscle type (*Longissimus lumborum* and *Semimembranosus*) on the content of vitamins and minerals in meat

| Parameter | LL | | | SM | | | SEM | P-value | | Inter-action |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | O | OS | C | O | OS | | diet | muscle | |
| Vitamins (mg 100 $g^{-1}$ of fresh meat) | | | | | | | | | | |
| A | $0.067^{a}$ | $0.066^{a}$ | $0.053^{b}$ | $0.058^{a}$ | 0.055 | $0.046^{b}$ | 0.002 | 0.048 | 0.064 | 0.946 |
| E | 0.158 | 0.139 | 0.140 | $0.129^{a}$ | $0.183^{b}$ | $0.202^{b}$ | 0.234 | 0.046 | 0.709 | 0.821 |
| Minerals (mg 100 $g^{-1}$ of fresh meat) | | | | | | | | | | |
| K | 472.3 | 490.8 | 478.8 | 498.3 | 507.5 | 505.0 | 4.101 | 0.350 | 0.056 | 0.844 |
| Na | $47.01^{a}$ | $49.93^{b}$ | $50.87^{b}$ | 47.93 | 48.91 | 50.22 | 0.509 | 0.047 | 0.805 | 0.700 |
| Mg | 22.20 | 23.03 | 22.38 | 21.79 | 22.45 | 21.51 | 0.231 | 0.305 | 0.189 | 0.920 |
| Fe | $1.214^{A}$ | $1.975^{B}$ | $1.871^{B}$ | 2.195 | 2.229 | 2.045 | 0.066 | 0.004 | 0.004 | 0.001 |
| Zn | $3.725^{a}$ | $4.056^{b}$ | $4.041^{b}$ | $4.199^{a}$ | $4.645^{b}$ | $4.709^{b}$ | 0.077 | 0.01 | 0.0003 | 0.809 |

LL – *Longissimus lumborum* muscle, SM – *Semimembranosus* muscle;

$^{A-B}$ Mean values in a row followed by different letters within the muscle are significantly different ($P<0.01$).

$^{a-b}$ Mean values in a row followed by different letters within muscle are significantly different ($P<0.05$).

Number of animals – $n=8$ per treatment.

control group than in experimental groups O and OS. A significant interaction between both experimental factors (dietary treatment x muscle type) was noted only for Fe. It was manifested in the fact that in response to both diets, the differences between the concentration of Fe, taking both muscles into account, were changing. Although Fe concentration increased in response to OS in both analyzed muscles ($P<0.05$), a higher value was noted in the SM muscle ($P<0.01$). A beneficial effect of the dietary treatments on Na concentration was noted only in the LL muscle.

**Fatty acid profile**

The concentrations of fatty acids, major fatty acid groups and ratios in the IMF of bulls are presented in Table 4. Fat content was affected by the diet ($P<0.01$), and muscle types differed in fat content ($P<0.01$). The fat content of LL and SM muscles was lower in treatment OS than in treatments C and O. The decrease in fat content in response to diet OS was greater in the SM muscle (0.822%). In the present study, UFAs were the predominant fatty acids in the IMF of LL and SM muscles. However, fat extracted from the SM muscle had higher concentrations of UFAs, in particular PUFAs, than the LL muscle. The total concentrations of SFAs as well as the concentrations of individual SFAs and anteiso fatty acids were highly significantly higher in the LL muscle. In this experiment, the concentration of CLA was not affected by the herbal extracts. Its level did not differ between LL and SM muscles.

Table 4

Effects of dietary supplementation with O and OS herbal preparations and muscle type (*Longissimus lumborum* and *Semimembranosus*) on the content of intramuscular fat, fatty acid groups, ratios and selected fatty acids profile

| Parameter | LL | | | SM | | | SEM | *P*-value | | Inter-action |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | O | OS | C | O | OS | | diet | muscle type | |
| Fat (%) | $3.281^{A}$ | $3.402^{A}$ | $2.621^{B}$ | $1.363^{a}$ | $1.572^{a}$ | $0.822^{b}$ | 0.231 | 0.000 | 0.015 | 0.421 |
| Main groups of the fatty acids (% of total fatty acids) | | | | | | | | | | |
| SFA | 46.60 | 43.64 | 44.56 | 41.46 | 40.96 | 41.28 | 0.437 | 0.117 | 0.000 | 0.310 |
| UFA | 53.40 | 56.36 | 55.44 | 56.33 | 56.70 | 56.21 | 0.357 | 0.142 | 0.053 | 0.253 |
| MUFA | 46.14 | 49.13 | 49.20 | 48.94 | 49.77 | 49.26 | 0.394 | 0.087 | 0.128 | 0.302 |
| PUFA | 7.261 | 7.235 | 6.238 | 9.513 | 9.276 | 9.460 | 0.271 | 0.539 | 0.000 | 0.460 |
| *n*-3 PUFA | $1.109^{A}$ | $1.087^{A}$ | $0.714^{B}$ | 1.087 | 1.109 | 1.087 | 0.031 | 0.002 | 0.016 | 0.004 |
| *n*-6 PUFA | $7.509^{A}$ | $7.73^{A}$ | $4.833^{B}$ | 7.698 | 7.509 | 7.730 | 0.250 | 0.017 | 0.027 | 0.00 |
| Ratios | | | | | | | | | | |
| MUFA/SFA | 1.002 | 1.128 | 1.106 | 1.187 | 1.220 | 1.199 | 0.019 | 0.145 | 0.00 | 0.433 |
| PUFA/SFA | 0.156 | 0.166 | 0.141 | 0.229 | 0.229 | 0.231 | 0.008 | 0.666 | 0.00 | 0.601 |
| *n*-6/*n*-3 PUFA | 6.756 | 7.097 | 6.783 | 7.127 | 6.756 | 7.097 | 0.104 | 0.989 | 0.595 | 0.330 |
| Saturated fatty acids (%) | | | | | | | | | | |
| C 14:0 | 2.46 | 2.33 | 2.67 | 1.98 | 1.91 | 1.90 | 0.06 | 0.43 | 0.00 | 0.34 |
| C 16:0 | $27.88^{a}$ | $25.19^{b}$ | 25.85 | 24.17 | 23.04 | 23.24 | 0.33 | 0.01 | 0.00 | 0.41 |
| C 18:0 | 14.41 | 14.22 | 14.22 | 13.57 | 14.18 | 14.42 | 0.18 | 0.76 | 0.55 | 0.49 |
| Unsaturated fatty acids (%) | | | | | | | | | | |
| C 14:1 | 0.484 | 0.491 | 0.608 | 0.352 | 0.339 | 0.313 | 0.024 | 0.589 | 0.000 | 0.205 |
| C 16:1 | 3.534 | 3.838 | 4.017 | 3.271 | 3.280 | 3.027 | 0.093 | 0.723 | 0.001 | 0.206 |
| C 18:1 T10+11 | 0.918 | 0.970 | 1.037 | 0.902 | 0.853 | 0.921 | 0.035 | 0.678 | 0.259 | 0.810 |
| C 18:1 C9 | 37.71 | 40.04 | 39.81 | 40.60 | 41.39 | 41.04 | 0.375 | 0.166 | 0.013 | 0.563 |
| C 18:1 C11 | 1.605 | 1.774 | 1.663 | 1.796 | 1.925 | 1.955 | 0.034 | 0.133 | 0.001 | 0.625 |
| C 18:2 | 4.559 | 4.501 | 3.914 | 6.025 | 5.906 | 5.999 | 0.176 | 0.566 | 0.001 | 0.513 |
| CLA | 0.292 | 0.328 | 0.327 | 0.307 | 0.304 | 0.292 | 0.006 | 0.524 | 0.146 | 0.066 |
| C 18:3 | 0.410 | 0.447 | 0.370 | 0.483 | 0.501 | 0.454 | 0.011 | 0.053 | 0.001 | 0.826 |
| C 20:4 | 1.190 | 1.103 | 0.919 | 1.674 | 1.603 | 1.731 | 0.071 | 0.734 | 0.000 | 0.426 |
| C 20:5 EPA | 0.097 | 0.095 | 0.069 | 0.130 | 0.140 | 0.138 | 0.006 | 0.413 | 0.000 | 0.282 |
| C 22:5 DPA | 0.307 | 0.292 | 0.244 | 0.423 | 0.418 | 0.446 | 0.017 | 0.835 | 0.000 | 0.364 |
| C 22:6 DHA | 0.048 | 0.039 | 0.030 | 0.051 | 0.049 | 0.049 | 0.002 | 0.166 | 0.011 | 0.315 |

LL – *Longissimus lumborum* muscle, SM – *Semimembranosus* muscle; SFA – saturated fatty acids; UFA – unsaturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; *n*-3 PUFA – ∑(C 18:2 Δ9c,12c; C 20:2 Δ11c,14c; C 20:4 Δ5c,8c,11c,14c);
*n*-6 PUFA – ∑(C 18:3 Δ9c,12c,15c; C 20:5 Δ5c,8c,11c,14c,17c; C 22:5 Δ7c,10c,13c,16c,19c; C 22:6 Δ4c,7c,10c,13c,16c,19c); CLA – C 18:2 C9 T11 conjugated linoleic acid;
$^{A-B}$ Mean values in a row followed by different letters within the muscle are significantly different ($P<0.01$);
$^{a-b}$ Mean values in a row followed by different letters within muscle are significantly different ($P<0.05$);
number of animals – $n=8$ per treatment.

The concentrations of *n*-3 and *n*-6 PUFAs (%) were affected by dietary treatments (interaction at $P \leq 0.01$), and they differed between the analyzed muscles. The LL muscle of group OS animals had the lowest PUFA content, which was reflected in the concentrations of *n*-3 and *n*-6 PUFAs (%). The *n*-6/*n*-3 PUFA ratio was not affected by the experimental factors. The values of the PUFA/SFA ratio, were low, in the range of 0.141 to 0.231.

## Biochemical profile of bovine serum

Active plant ingredients significantly affected the levels of selected biochemical serum parameters (Table 5). Differences were found in the activity of indicator enzymes (ALT, AST and ALP) and in the concentrations of TP, CHOL, GLU and UREA. Decreased activity of ALT and AST and increased activity of ALP were noted in groups O and OS. Serum UREA levels were higher in group OS and serum GLU levels were higher in both dietary treatment groups than in the control group (C). A different relationship was noted for serum CHOL levels, which decreased at the end of the experimental fattening period. The values of most biochemical serum parameters (TP, CHOL, GLU, UREA, TG, CREA, LDH-L) remained within the reference ranges given in the literature (Meyer, Harley 2004, Winnicka 2008).

Table 5

Effects of dietary supplementation with O and OS herbal preparations and the date of blood sampling on the biochemical profile of bovine serum

| Parameter | Experimental day 1 | | | Slaughter day | | | SEM | P-value | | Interaction |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | O | OS | C | O | OS | | date | diet | |
| ALT | 22.56 | 20.74 | 22.98 | $22.16^{A}$ | $13.39^{B}$ | $13.66^{B}$ | 0.760 | 0.000 | 0.000 | 0.001 |
| AST | 63.93 | 58.77 | 65.20 | $65.22^{Aa}$ | $45.43^{B}$ | $48.52^{b}$ | 1.750 | 0.001 | 0.002 | 0.020 |
| ALP | 168.4 | 126.7 | 125.6 | $160.3^{A}$ | $227.5^{B}$ | $200.9^{B}$ | 9.060 | 0.001 | 0.713 | 0.017 |
| LDH-L | 903.2 | 961.1 | 921.7 | 931.8 | 866.8 | 893.2 | 10.89 | 0.146 | 0.923 | 0.080 |
| TP | 6.331 | 6.940 | 6.392 | 6.219 | 5.639 | 6.190 | 0.120 | 0.020 | 0.999 | 0.067 |
| CHOL | 101.5 | 87.30 | 89.40 | $95.62^{A}$ | $79.84^{b}$ | $78.63^{b}$ | 1.830 | 0.014 | 0.000 | 0.693 |
| GLU | 56.22 | 59.18 | 62.99 | $54.72^{A}$ | $68.74^{B}$ | $63.91^{B}$ | 0.950 | 0.029 | 0.000 | 0.005 |
| UREA | 9.350 | 11.36 | 10.51 | $9.060^{a}$ | 13.70 | $14.45^{b}$ | 0.520 | 0.029 | 0.005 | 0.152 |
| TG | 18.00 | 18.42 | 19.95 | 20.69 | 17.91 | 17.93 | 0.500 | 0.206 | 0.026 | 0.048 |
| CREA | 1.209 | 1.121 | 1.140 | 1.170 | 1.230 | 1.173 | 0.010 | 0.170 | 0.584 | 0.054 |

ALT – alanine aminotransferase (U $L^{-1}$); AST – aspartate aminotransferase (U $L^{-1}$); ALP – alkaline phosphatase (U $L^{-1}$); LDH-L – lactate dehydrogenase (U $L^{-1}$); TP – total protein (g $dL^{-1}$); CHOL – cholesterol (mg $dL^{-1}$); GLU – glucose (mg $dL^{-1}$); UREA – urea (mg $dL^{-1}$); TG – triglycerides (mg $dL^{-1}$); CREA – creatinine (mg $dL^{-1}$);

$^{A-B}$ Mean values in a row followed by different letters within the muscle are significantly different ($P<0.01$).

$^{a-b}$ Mean values in a row followed by different letters within muscle are significantly different ($P<0.05$).

Number of animals – $n$=8 per treatment.

# DISCUSSION

## Carcass traits

The results of the present experiment are consistent with the conclusions of other authors (Rivaroli et al. 2017, Zawadzki et al. 2017, Ornaghi et al. 2020, Wilson et al. 2020) who demonstrated that essential oils and natural additives had no effect on performance or carcass characteristics in beef cattle and cattle of different sex categories.

## Vitamins and minerals

Attempts have been made to improve beef quality by modifying the amount of dietary vitamin A during fattening. Vitamin A was found o be negatively correlated with IMF content in beef cattle (Chae et al. 2003, Moon et al. 2018). Farther-reaching conclusions were drawn by researchers from the Ohio State University (Gorocica-Buenfil et al. 2007, 2008), who reported that dietary vitamin A restriction in beef cattle increased IMF content and improved meat quality. The relationships observed in the cited study were not confirmed in the present experiment. Muscle tissue must contain a minimum concentration of antioxidants, such as vitamin E, in order to stabilize high concentrations of PUFAs, prevent lipid oxidation, reduce drip loss and metmyoglobin formation (Amaral et al. 2018). Lipid oxidation is one of the key factors responsible for a gradual decrease in the sensory quality and nutritional value of meat, which compromises consumer acceptance (Scollan et al. 2014). This process can be controlled with different strategies, such as the addition of antioxidants (Li, Liu 2012), the use of various processing methods (Min and Ahn 2005), special packaging (Pereira et al. 2015) and dietary supplements (Scollan et al. 2014). Feedstuffs with low vitamin A levels usually have also low vitamin E levels, and the produced beef may be low in vitamin E (Irie et al. 2006). Polyphenols present in herbs can maintain high tocopherol levels in lipid structure either by sparing it or by recycling its oxidized form. Research has also shown that tocopherol rapidly leaves the plasma, but it is also returned to the plasma pool from the diet or tissue reserves (Bhatt 2015).

Natural vitamin E occurs in eight isoforms, of which alpha-tocopherol is characterized by the highest bioactivity in vivo (Raederstorff et al. 2015). Liu et al. (2011) found that alpha-tocopherol accounted for 79% of the variation in lipid stability (TBARS) and that the optimal alpha-tocopherol concentration for antioxidant capacity was 3.0 to 3.5 mg $kg^{-1}$ of tissue. Higher alpha-tocopherol concentrations did not affect antioxidant capacity. In the current study, vitamin E concentration in the SM muscle was considerably lower than that noted in previous studies. Modzelewska-Kapituła et al. (2018) found that TBARS values, an indicator of lipid oxidation in meat, were not affected by the diet and did not differ between muscle types.

This could result from the low levels of the analyzed vitamins in both muscles (vitamin A – 0.05 to 0.07 mg 100 $g^{-1}$ fresh meat, vitamin E – 0.14 to 0.20 mg 100 $g^{-1}$ fresh meat). At excessive doses of antioxidants, active ingredients can act as pro-oxidants (Rivaroli et al. 2016).

Beef, among other commonly consumed meats, is considered to be the richest source of highly bioavailable macrominerals and microminerals in the diet, in particular Fe and Zn (Czerwonka, Szterk 2015). The content and bioavailability of microminerals may vary widely across meat cuts (Ramos et al. 2012). In the current study, Fe concentration was lower than in a previous study investigating the Fe content of the *Longissimus dorsi* muscle (Momot et al. 2016), where Fe levels remained in the range of 1.87 to 1.94 mg 100 $g^{-1}$ of raw meat regardless of the diet (semi-intensive and intensive production systems). Czerwonka and Szterk (2015) reported Fe concentration of 1.83 mg 100 $g^{-1}$ of meat in HF bulls slaughtered at 18 to 22 months of age, housed and fed diets produced in Central-Eastern Europe. Iron levels in pasture and its bioaccessibility during digestion (Ramos et al. 2012) should be taken into account when comparing the results of studies conducted in different regions of the world. The higher concentration of Fe in the SM muscle (2.045 to 2.229 mg 100 $g^{-1}$) than in the LL muscle, noted in the current study, is consistent with the findings of Czerwonka and Szterk (2015) who also found that the SM muscle had higher Fe content than the *Longissimus dorsi* muscle. The differences in mineral concentrations between different muscles in European cattle breeds may result from differences in the metabolic activity of animals, fat content and blood supply to internal organs (López-Alonso et al. 2000, García-Vaquero et al. 2011). The concentrations of trace and essential elements vary across muscle types. The levels of essential trace elements (Fe and Zn) are higher in muscles with a high proportion of oxidative slow-twitch fibers (red muscles). In turn, the concentrations of trace elements are lower in muscles with a high proportion of glycolytic fast-twitch fibers (white muscles) (López-Alonso et al. 2016, Miranda et al. 2018). If the mineral status of an animal is adequate, it has no effect on the concentrations of essential trace elements in muscles, which appear to be associated with the metabolism of individual muscles (Fernandez-Garcia et al. 2009) According to Nohr and Biesalski (2007), Fe has higher bioavailability when derived from meat as hem iron, compared with plant-derived non-hem iron. Low or no intake of meat poses a risk of inadequate Fe supply and development of deficiency symptoms. Zinc is essential for the activity of more than 100 enzymes, and it plays an important role in the immune system (Nohr, Biesalski 2007). In the present study, the Zn content of the analyzed muscles remained within the reference ranges included in the USDA (2020) and NORFOODS (2019) nutrient databases: 3.2 to 7.9 mg 100 $g^{-1}$ of raw meat and 1.0 to 5.1 mg 100 $g^{-1}$ of raw meat, respectively. Trace element concentrations in meat do not depend only on the dietary intake but may also be related to the metabolic capacity of the animal to deliver trace elements from the liver to the muscle (Pereira et al.

2018). In cattle, Zn tissue concentrations are efficiently regulated by homeostatic mechanisms and, once optimal physiological values are reached, Zn supplementation has no significant effect on Zn muscle levels (Kessler et al. 2003). Some essential metals, including Zn, do not follow the same intermuscular distribution pattern, which could be related to metal interactions or antagonisms to maintain a proper mineral balance. Other dietary components may influence the distribution of Zn in the body. For example, elements such as Cu and Cd have similar chemical and physical properties to Zn and compete for metabolic binding sites in metallothioneins (López-Alonso et al. 2002). The differences in the trace element content between muscles are difficult to interpret due to the lack of studies involving various metals and muscle types, since most research efforts have been focused on carcass quality or nutritional composition.

**Fatty acid profile**

The applied dietary treatments had a beneficial effect on reducing the concentration of C 16:0 palmitic acid (the predominant SFA) in the LL muscle. Other major SFAs in beef are C 18:0 stearic acid and C 14:0 myristic acid. Palmitic acid and myristic acid, but not stearic acid, are associated with elevated plasma levels of total CHOL and LDL-C (Williams 2000). The tested herbal extracts had a minor effect on the fatty acid profile of IMF, which corroborates previous findings (Rivaroli et al. 2016, Monteschio et al. 2019). The above can be due to the fact that all animals were fed identical basal diets that did not differ in fat content or profile. Nine detected and determined functional fatty acids deliver health benefits: vaccenic acid (C 18:1 T10+11), oleic acid (C 18:1 C9), linoleic acid (C 18:2), CLA (C 18:2 C9 T11), alpha-linolenic acid (C 18:3), arachidonic acid (C 20:4), EPA (C 20:5), DPA (C 22:5) and DHA (C 22:6). In this group, the percentage of oleic acid was the highest (37.71% to 41.39%), which is consistent with the results of earlier studies (Rivaroli et al. 2016, Beyzi et al. 2019). Oleic acid exerts anti-atherosclerotic (an increase in HDL-C concentration and a decrease in LDL-C concentration) and anti-thrombotic (a decrease in platelet aggregation) effects, and reduces systemic inflammation (Rodrigues et al. 2010). Rivaroli et al. (2017) demonstrated that essential oils added to diets for crossbred bulls had a minimal effect on the proportion of oleic acid in fat extracted from the Longissimus thoracis muscle.

Some tannins and phenolic compounds can modulate ruminal biohydrogenation because they selectively inhibit the growth of bacteria such as *Fusocillus spp.* and *Clostridium proteoclasticum* which convert vaccenic acid to stearic acid, thus increasing the amount of vaccenic acid for conversion to CLA in animal tissues. The above compounds can also alter the fatty acid composition of muscles. Cis-9 trans-11 is the most prevalent CLA isomer, accounting for 80 to 90% of the total CLA in ruminant tissues (Azain 2003). In the current experiment, its content ranged from 0.292% to 0.329%.

In a study by Zawadzki et al. (2017), an increase in the concentration of mate extract in cattle diets led to an increase in CLA concentration in muscles. Monteschio et al. (2019) demonstrated that the dietary inclusion of essential oils exerted no significant effects, and biohydrogenation intermediates, in particular vaccenic acid, were the most variable fatty acids, relative to their average content, which strongly suggested that no modulatory effects on rumen biohydrogenation had occurred. The absence of effects of herbal extracts on the concentrations of vaccenic acid and CLA, corroborates the findings of Zawadzki et al. (2017). The CLA content remained within the normal range for beef cattle (0.2 to 1.0% of total fatty acids), according to Raesa et al. (2003).

The results of experimental studies suggest that the optimal *n*-6/*n*-3 PUFA ratio should be close to 4:1 to 5:1, and it should not exceed 10:1 (Gómez Candela et al. 2011). In the present study, the *n*-6/*n*-3 PUFA ratio remained within the above range, and it was not affected by the experimental factors. A high dietary *n*-6/*n*-3 PUFA ratio may be a risk factor for prostate cancer, chronic inflammatory disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis and Alzheimer's disease (Raederstorff et al. 2015). In the current experiment, the SM muscle had higher ($P \leq 0{,}01$) linoleic acid content than the LL muscle in all dietary treatments.

Despite less intensive fattening in all groups, the PUFA/SFA ratio was lower than the minimal value of 0.45 recommended by the UK Department of Health (1994). Such low values of the PUFA/SFA ratio (0.14 to 0.23) are typical of meat from animals raised under intensive production systems. Rivaroli et al. (2016) reported that dietary supplementation with essential oils had no influence on the chemical composition or fatty acid profile of meat from young crossbred bulls.

**Biochemical profile of bovine serum**

Plants may exert multiple effects on the gastrointestinal function. The lower activity of ALT and AST in groups O and OS indicates that the herbal blends exerted a positive, protective effect on liver function and structure, and suggests that animals were not exposed to severe stress. The above findings are consistent with the results of a previous study where high blood AST, total CHOL and TG levels in the finishing phase were correlated with a burden on the liver and IMF accumulation in Hanwoo steers. The aspartate aminotransferase concentration was also negatively correlated with the rib-eye muscle area, marbling score and meat quality grade (Moon et al. 2018). Alkaline phosphatase is present in many tissues, but two ALP isoforms, skeletal and hepatic, predominate in the serum (Szutowicz, Raszei-Specht 2009). Enhanced activity of ALP, in contrast to the levels of ALT and AST, may suggest disorders of calcium and vitamin $D_3$ metabolism, which requires further diagnostics. However, in most species, elevated serum levels

of ALP have been associated with cholestasis as well as hepatocyte injury. Elevated serum ALP activity may also be caused by an increase in the number of osteoblasts in the bones of young growing calves, which is less alarming (Szutowicz, Raszei-Specht 2009). Serum UREA concentration can be used for estimating the utilization efficiency of dietary protein. In the present study, serum UREA levels were higher in group OS than in the control group (C), but they remained within the reference range, which indicates that the effective ruminal degradability of protein was adequate. The herbal blends tested in the present study contributed to lowering serum CHOL levels, which could be due to the presence of plant species with anti-hyperlipidemic activity in both blends, such as marking nut tree (*Semecarpus anacardium*), coriander (*Coriandrum sativum*), garlic (*Allium sativum*), ginger (*Zingiber officinale*) and ashwagandha (*Withania somnifera*) – Dwivedi et al. (2014).

The observed increase in GLU levels in groups O and OS ($P \leq 0.01$) could have been caused by an increase in the concentration of rumen propionate, which is a precursor for GLU synthesis. In a study by Hosoda et al. (2006), the rumen propionate concentration decreased in Holstein steers fed clove-supplemented diets and did not decrease in those receiving peppermint and lemongrass. It appears that the active constituents of herbs may affect rumen fermentation and microbial activity in ruminants.

## CONCLUSIONS

In the present study, the herbal extracts contributed to an increase in the concentrations of Na, Fe and Zn in both analyzed muscles, but the SM muscle had higher levels of Fe and Zn. The SM muscle was more susceptible to dietary modifications than the LL muscle, and it was characterized by a lower content of fat with a more nutritionally desirable profile, and a higher vitamin E concentration. The biochemical profile of bovine serum being within the norms proved the proper functioning of the gastrointestinal tract. The results of this study suggest that more complex herbal preparations have a more beneficial impact on meat quality, therefore composite herbal preparations can be recommended as feed additives in cattle production.

### Conflicts of interest

The authors declare no conflict of interest.

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