

## Article

# Assessing the Impact of Sustainable Pasture Systems on Lamb Meat Quality

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**Abstract:** The global demand for sustainable lamb production is increasing due to the need for high-quality meat with minimal environmental impact, making the choice of feeding systems crucial. This study investigates the effects of supplemented pasture feeding during the last 60 days of rearing on the meat fatty acid profile, pH value, colour characteristics, and mineral composition of lambs, highlighting the benefits of such feeding systems. Ninety lambs (MIS sheep breed) were divided into three distinct feeding regimes: Group I (alfalfa and concentrate feeding), Group II (white clover [*Trifolium repens*] pasture with concentrate supplementation), and Group III (birds' foot trefoil [*Lotus corniculatus*] pasture with concentrate supplementation). The results have shown that supplemented pasture feeding improves the fatty acid profile by increasing n-3 content and desirable fatty acids, while reducing the n-6/n-3 ratio and atherogenic index ( $p < 0.05$ ), particularly in lambs finished on an *L. corniculatus* diet. However, forage-supplemented feeding also reduces meat colour lightness and redness ( $p < 0.05$ ). On the other hand, it enhances the meat's mineral profile, with higher calcium, selenium, and iron levels, especially in lambs fed *L. corniculatus*. These findings underscore the benefits of moderate grazing with supplemental concentrates in optimising lamb meat quality. Importantly, they also highlight the potential of forage legumes like *T. repens* and *L. corniculatus* to significantly enhance the nutritional profile of lamb meat, offering a promising outlook for the future of sustainable lamb production. Additionally, this research provides valuable insights that could guide the development of future agricultural practices, dietary guidelines, and environmental policies to advance sustainable and nutritious food systems.

**Keywords:** supplemented pasture system; lamb meat quality; fatty acid composition; instrumental colour; mineral composition



**Citation:** Stanišić, N.; Ružić-Muslić, D.; Maksimović, N.; Cekić, B.; Caro Petrović, V.; Ćosić, I.; Lazarević, M. Assessing the Impact of Sustainable Pasture Systems on Lamb Meat Quality. *Processes* **2024**, *12*, 1532. <https://doi.org/10.3390/pr12071532>

Academic Editor: Nevijo Zdolec

Received: 23 June 2024  
Revised: 11 July 2024  
Accepted: 18 July 2024  
Published: 20 July 2024



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## 1. Introduction

The global lamb meat market is projected to grow at a compound annual growth rate (CAGR) of around 3.5% from 2023 to 2030, driven by increasing consumer demand for high-quality protein and expanding middle-class populations in emerging markets [1]. This increase in global demand for sustainable practices in the lamb industry is primarily driven by the need for high-quality meat with minimal environmental impact [2]. In Serbia, lamb farming relies on abundant natural pastures during spring and summer, providing a rich diet that enhances meat quality, while dried grass or hay is used in winter when fresh grass is unavailable. The country has diverse grasslands that support extensive sheep farming, primarily in hilly and mountainous regions.

The choice of feeding systems plays a crucial role in determining the quality, consumer acceptance, and sustainability of lamb meat [3]. Studies have shown that enhancing production through concentrate feeding is less efficient and more detrimental to the environment compared to pasture feeding [4]. Grazing outdoors is not only cost-effective but also

has better social acceptance due to its association with natural attributes, environmental care, and animal welfare [5,6]. Forage-based systems are promising, as they can reduce animal fat content, lower production costs, and make better use of resources, aligning with consumer preferences for high-quality meat [7]. Commonly cultivated pastures include grasses (e.g., *Agrostis* spp., *Lolium* spp., *Festuca* spp., and *Dactylis* spp.) and herbaceous legumes (e.g., *Medicago* spp., *Lotus* spp., and *Trifolium* spp.) [8]. However, while pasture feeding improves the nutritional and technological aspects of meat, it can result in darker meat with stronger, sometimes off-putting flavours and inconsistent carcass fatness [9]. Additionally, overgrazing can also diminish grassland productivity and disrupt ecological balance, particularly in arid regions [10].

Recent reviews highlight the impact of grazing on improving lamb meat quality, especially its fatty acid composition [9,11]. Conversely, concentrate feeding enhances weight gain and carcass weight, but compromises meat quality by increasing saturated fats, raising health concerns [12]. This presents a trade-off between higher production from indoor feeding and superior meat quality from grazing [2]. A combined approach of grazing with grain supplementation or stall finishing could balance meat quality and production efficiency [2,13]. Research indicates that moderate grazing supplemented with appropriate concentrates optimises lamb growth and grassland productivity [10,14]. Supplemental feeding improves weight gain, carcass characteristics, and meat quality in lambs on pasture or roughage diets [15–17]. Lambs grow significantly faster with a pasture plus concentrate supplement than with pasture alone [18]. Aguayo-Ulloa et al. [19] reported that pasture-based finishing systems are a viable, cost-effective alternative to feedlot finishing.

Forage legumes like red clover (*Trifolium pratense*) and lucerne (*Medicago sativa*) are well documented as effective protein supplements to grass silage, with high intake potential linked to increased animal performance [20,21]. In contrast, despite their reported potential in ruminant feeding, forages like white clover (*Trifolium repens*) and birds' foot trefoil (*Lotus corniculatus*) have been investigated less regarding their effect on meat quality characteristics. In the context of sustainability, they are significant forages with distinct ecological advantages. White clover, renowned for its high nutritional value and phenolic compounds, contributes to enhanced animal performance and protein utilisation. Its enzyme, polyphenol oxidase, aids in reducing proteolysis, thereby increasing protein availability from silage and rumen digestion [22]. Furthermore, *L. corniculatus*, also known as "birds' foot trefoil", enhances essential amino acid absorption and milk protein synthesis in ruminants [23,24]. It is worth mentioning that *L. corniculatus* is a significant source of tannins, which can positively affect protein utilisation and, consequently, the daily weight gain of grazing lambs [25,26]. Both forages offer sustainable alternatives by improving grazing efficiency and nutrient utilisation, and potentially reducing the environmental impact associated with intensive feeding practices.

Fresh grass provides lambs with high moisture content, vitamins, and minerals essential for growth, but its availability is limited by seasonal and weather conditions. In contrast, dried grass, or hay, offers a consistent and convenient feed option that can be stored for long periods, though it loses some vitamins during drying [20,21]. Both fresh grass and hay are valuable in lamb diets, with fresh grass being richer in certain nutrients and hay providing a reliable year-round food source [9]. The use of fresh, high-quality pastures in intensive animal production systems is globally recognised as economically advantageous, environmentally friendly, and supportive of animal welfare. However, while the combination of high-quality pastures and concentrate feeding is well-documented for dairy cow grazing systems, there is less information available for sheep [27]. Furthermore, the effects of concentrate-supplemented grazing systems on lamb meat quality are not thoroughly explained in the literature due to variability in factors such as sex, age, breed, season, and forage source [28]. This study aims to elucidate these effects, focusing on the meat fatty acid profile, mineral composition, and colour attributes of lambs finished on two

types of supplemented forage grazing: white clover (*Trifolium repens*) and birds' foot trefoil (*Lotus corniculatus*).

## 2. Materials and Methods

### 2.1. Animals and Experimental Design

The study protocol was approved and conducted following the Animal Ethics Committee guidelines of the Institute for Animal Husbandry (Belgrade, Serbia), decision number 323-07-07552/2020-05.

This study was conducted at the Institute for Animal Husbandry in Belgrade, Serbia (44°49' N, 20°17' E, with an elevation of 96 m a.s.l.) with MIS lambs. This breed was chosen based on the criterion of being a common breed and well adapted to the local environmental conditions. MIS sheep is a meaty breed formed by crossbreeding of Pirot pramenka (a local indigenous breed), Merinolandschaf, and Île-de-France [29]. Lambs were kept together with their mothers in pens after birth, where they were continuously provided with alfalfa hay and commercial lamb grower feed. When their age reached 60 days (23.0 kg of body weight on average), only male lambs were chosen and randomly divided into three production systems (30 lambs each): concentrate-based and two supplemented pasture-based systems. Lambs in a concentrate-based production system (Group I) were kept in pens throughout the study and consumed 0.5 kg of alfalfa hay and 0.5 kg of commercial concentrated feed per animal, daily. Lambs in pasture production systems were taken to pasture between 07:30 and 17:00 every day (from mid-June to mid-July in 2020), while at night, they were kept at sheepfold. The lambs in two supplemented pasture-based systems received 0.5 kg of hay (in the morning), 0.3 kg of concentrated mixture (in the evening), and 3.0 kg of green mass from pasture per animal, as a daily meal. Lambs in Group II received white clover (*Trifolium repens*) as a leguminous component, while the lambs in Group III consumed birds' foot trefoil (*Lotus corniculatus*). All the plants were in a pre-bloom vegetative state. The feeds and composition of rations are shown in Table 1.

**Table 1.** Ingredients and chemical composition of rations.

Items	Group I	Group II	Group III
Ingredients (kg/day)			
Hay (alfalfa)	0.50	0.50	0.50
Concentrate feed <sup>1</sup>	0.50	0.30	0.30
Biomass (pasture yield) <sup>2</sup>	-	3.00	3.00
Chemical composition of ration (% DM) <sup>3</sup>			
CP	16.00	16.60	15.80
ADF	25.20	26.00	25.80
NDF	41.95	40.72	41.03
Ash	8.43	9.21	8.88
Energy value (MJ/day)	7.53	7.50	7.62

<sup>1</sup> Maize (67%), sunflower meal (16%), soybean meal (7%), wheat bran (6%), chalk (2%), vitamin/mineral premix (1%), and salt (1%); <sup>2</sup> Group I—control group; Group II—white clover (*Trifolium repens*); Group III—birds' foot trefoil (*Lotus corniculatus*). <sup>3</sup> DM—dry matter of ration; CP—crude protein; ADF—acid detergent fibre; NDF—neutral detergent fibre; Ash—crude ash.

### 2.2. Slaughter and Sampling

After a 60-day feeding trial, all lambs were transported from the farm to the Institute for Animal Husbandry slaughterhouse, which is 500 m away. The lambs were slaughtered within 24 h of leaving the farm, following the standard practice for commercial processing. While in lairage, the lambs had access to water. They were electrically stunned (50 Hz, 2 s, 1 A), exsanguinated, and dressed according to standard commercial procedures. The carcasses were chilled at 0 °C for 24 h.

Following chilling, samples of the *Longissimus thoracis et lumborum* were collected from each left carcass for analysis. Any visible fat and connective tissues were diligently removed,

and the dorsal part of the muscle was taken for instrumental colour determination. The remaining samples were sealed in plastic bags under vacuum and stored at  $-20\text{ }^{\circ}\text{C}$  until further chemical analyses. Each meat sample was then homogenised in a laboratory mixer (CombiMax 600, Braun, Germany) before undergoing thorough analysis.

### 2.3. Fatty Acids Analyses

Fatty acid methyl esters (FAMES) were prepared by direct transesterification following the method outlined by O'Fallon et al. [30]. In brief, a 1 g meat sample was placed into a 15 mL tube and treated with 0.7 mL of 10 N KOH in water and 6.3 mL of methanol. The tube was then incubated in a  $55\text{ }^{\circ}\text{C}$  water bath for 1.5 h, with intermittent shaking every 20 min. After cooling in a cold-water bath, 0.58 mL of 24 N  $\text{H}_2\text{SO}_4$  in water was added, followed by another incubation at  $55\text{ }^{\circ}\text{C}$  for 1.5 h with periodic shaking. Once the FAME synthesis was complete, the tube was cooled again in a cold tap water bath, and 3 mL of hexane was added. The organic phase, which contained the FAMES, was separated and prepared for gas chromatography (GC) analysis.

The determination of FAMES was carried out using a Shimadzu 2014 GC instrument (Kyoto, Japan). This instrument was equipped with a split/splitless injector, a fused silica cyanopropyl HP-88 column (60 m length, 0.25 mm internal diameter, 0.20  $\mu\text{m}$  film thickness), and a flame ionisation detector (FID). The column temperature was programmed, with the injector and detector maintained at  $260\text{ }^{\circ}\text{C}$  and  $280\text{ }^{\circ}\text{C}$ , respectively. Helium was used as the carrier gas at a flow rate of 1.0 mL/min, with an injector split ratio of 1:10. A volume of 1  $\mu\text{L}$  was injected. The GC oven programme initiated at  $50\text{ }^{\circ}\text{C}$  (held for 2 min), then increased at  $20\text{ }^{\circ}\text{C}/\text{min}$  to  $190\text{ }^{\circ}\text{C}$ , followed by a rise at  $10\text{ }^{\circ}\text{C}/\text{min}$  to  $200\text{ }^{\circ}\text{C}$  (held for 10 min), and, finally, at  $15\text{ }^{\circ}\text{C}/\text{min}$  to  $250\text{ }^{\circ}\text{C}$  (held for 2 min). Chromatographic peaks were identified by comparing the relative retention times of FAME peaks with those in a Supelco 37 Component FAME mix standard (Sigma-Aldrich, Hamburg, Germany) and individual fatty acids. The concentration of each fatty acid was reported as a percentage of the total fatty acids.

Lipid quality ratios relevant to human health were calculated as follows: desirable fatty acids (MUFA + PUFA + 18:0) [31], the n-6/n-3 PUFA ratio (PUFA/SFA), and the atherogenic index (AI:  $(12:0 + 4 \times 14:0 + 16:0)/(n-6 + n-3 + \text{MUFA})$ ) [32]. The delta-9 desaturase activity index for 18:0 was determined using the ratio  $18:1/(18:0 + 18:1)$  [33].

### 2.4. pH Value Analyses

The pH measurement was conducted 24 h post-slaughter using a Hanna pH metre (model HI 83141, Hanna Instruments, Smithfield, RI, USA) fitted with a puncture electrode. Calibration of the pH metre was performed following ISO 2917 [34] guidelines using standard phosphate buffers.

### 2.5. Instrumental Colour Analyses

Colour measurements were performed using a Chromameter CR-400 (Minolta Co. Ltd., Tokyo, Japan), configured with a D65 light source, a  $10^{\circ}$  observer angle, and an 8 mm aperture size. Calibration was executed using a white ceramic tile, and measurements adhered to the CIE Lab\* system, evaluating parameters such as lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ). Chroma ( $C^*$ ) and Hue angle (h) were computed using dedicated software. These assessments were conducted at room temperature ( $20 \pm 2\text{ }^{\circ}\text{C}$ ) on fresh meat samples following a 30 min blooming period.

### 2.6. Mineral Analyses

Mineral isotopic analysis of sodium ( $^{23}\text{Na}$ ), magnesium ( $^{24}\text{Mg}$ ), potassium ( $^{39}\text{K}$ ), calcium ( $^{44}\text{Ca}$ ), iron ( $^{57}\text{Fe}$ ), zinc ( $^{66}\text{Zn}$ ), and selenium ( $^{77}\text{Se}$ ) was performed using an "iCap Q" ICP-MS system (Thermo Scientific, Bremen, Germany) equipped with a collision cell operating in kinetic energy discrimination (KED) mode to minimise polyatomic-based Ar<sub>2</sub> interferences. Approximately 0.3 g of homogenised meat samples were placed into Teflon

vessels, and 5 mL of nitric acid (67% Trace Metal Grade, Fisher Scientific, Loughborough, UK) and 1.5 mL of hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MO, USA) were added for digestion. Microwave digestion was carried out using a Start D microwave (Milestone, Sorisole, Italy) with a program ramping from room temperature to 180 °C over 5 min, followed by a 10 min hold at 180 °C and a 20 min vent. After cooling, digested solutions were transferred to disposable flasks and diluted to 100 mL with deionised water produced by the Purelab DV35 purification system (ELGA, Lane End, UK).

Operating conditions for the instrument included a RF power of 1550 W, a cooling gas flow of 14 L/min, a nebulizer flow of 1 L/min, a collision gas flow of 1 mL/min, and a dwell time of 10 ms. The qualitative analysis utilised standard solutions from VGH Labs (Manchester, UK), all prepared in 2% nitric acid. A multielement internal standard (6Li, 45Sc-10 ng/mL; 71Ga, 89Y, 209Bi-2 ng/mL) was introduced online via an additional peristaltic pump line to cover a wide mass range. To ensure analytical accuracy and quality, standard reference material SRM 1577c (NIST, Gaithersburg, MD, USA) was included in the analysis. Mineral content was reported as mg/100 g of dry meat samples.

### 2.7. Statistical Analyses

A one-way analysis of variance (ANOVA) was performed using IBM SPSS Statistics, Version 20 software to assess the impact of various feeding regimes on meat quality parameters. Significant differences among the three groups were determined using Duncan's post hoc multiple comparisons test. The results were reported as means  $\pm$  standard deviation and statistical significance was defined at  $p < 0.05$ .

## 3. Results

### 3.1. Fatty Acids Composition

The *longissimus thoracis et lumborum* fatty acid profiles exhibited significant differences among the lamb groups (Table 2). While the supplemented pasture feeding system did not significantly affect the total saturated fatty acid (SFA) content, individual SFAs varied between groups. Notably, stearic acid (C18:0) was significantly higher in lambs from Group III compared to Group I ( $p = 0.042$ ). Additionally, lambs fed with the addition of white clover showed higher levels of decanoic (C10:0) and myristic (C14:0) acids than those fed with *L. corniculatus*.

The monounsaturated fatty acid (MUFA) content was significantly higher in the meat of lambs fed on supplemented *L. corniculatus* pasture than those kept indoors ( $p = 0.042$ ). This increase was primarily attributed to higher oleic acid (C18:1 n-9) levels in Group III compared to Group I, with Group II showing no significant difference ( $p = 0.041$ ). Group III also had higher levels of beneficial polyunsaturated fatty acids (PUFA) compared to the other groups ( $p = 0.039$ ), although linoleic acid (C18:2 n-6) content did not differ significantly between groups. Lambs kept indoors (Group I) exhibited lower levels of  $\alpha$ -linolenic (C18:3 n-3) and eicosatrienoic (C20:3 n-3) acids in their meat. Furthermore, lambs in Group III had a higher proportion of docosapentaenoic acid (C22:5 n-3) compared to those in Group II ( $p = 0.029$ ).

The fatty acid health indices of the *longissimus thoracis et lumborum* muscle revealed significant differences between lambs kept indoors and those under grazing supplementary feeding systems (Table 3). Group III exhibited a significantly higher sum of all C18:1 fatty acids than Group I ( $p = 0.043$ ). The total n-6 fatty acids content remained consistent across groups, as expected since individual n-6 fatty acid levels were unaffected by the feeding regime (Table 2). However, supplementation with *L. corniculatus* proved to be an excellent source of n-3 fatty acids, resulting in Group III lambs having a significantly higher proportion of total n-3 in their intramuscular fat ( $p = 0.014$ ). Consequently, the n-6/n-3 ratio was significantly lower in Group III ( $p = 0.035$ ), and these lambs also demonstrated a higher PUFA/SFA ratio compared to the other groups ( $p = 0.039$ ).

**Table 2.** Fatty acids composition of intramuscular fat of lambs under three feeding regimes.

Fatty Acids (%)	Group I	Group II	Group III	p-Value
C10:0	1.11 ± 0.24 <sup>ab</sup>	1.31 ± 0.37 <sup>b</sup>	0.62 ± 0.25 <sup>a</sup>	0.037
C12:0	0.91 ± 0.26 <sup>b</sup>	1.01 ± 0.47 <sup>b</sup>	0.51 ± 0.21 <sup>a</sup>	0.050
C14:0	3.33 ± 0.57 <sup>ab</sup>	4.60 ± 1.02 <sup>b</sup>	3.07 ± 0.99 <sup>a</sup>	0.019
C16:0	20.87 ± 0.93	20.86 ± 1.96	19.89 ± 3.01	0.693
C16:1 n-7	1.25 ± 0.43	1.47 ± 0.66	1.24 ± 0.15	0.281
C17:0	0.64 ± 0.34	0.78 ± 0.26	1.03 ± 0.56	0.304
C17:1 n-7	0.52 ± 0.36	0.36 ± 0.28	0.43 ± 0.17	0.479
C18:0	16.19 ± 1.58 <sup>a</sup>	17.83 ± 1.63 <sup>ab</sup>	19.10 ± 1.81 <sup>b</sup>	0.042
C18:1 n-9	30.55 ± 2.64 <sup>a</sup>	33.18 ± 2.44 <sup>ab</sup>	35.75 ± 2.81 <sup>b</sup>	0.041
C18:1 n-11	1.24 ± 0.14 <sup>a</sup>	1.71 ± 0.23 <sup>b</sup>	1.81 ± 0.17 <sup>b</sup>	0.020
C18:1 n-12	0.44 ± 0.21	0.42 ± 0.12	0.32 ± 0.22	0.551
C18:2 n-6	8.21 ± 1.27	8.18 ± 1.55	8.85 ± 3.03	0.836
C18:3 n-3	1.93 ± 0.31 <sup>a</sup>	2.30 ± 0.19 <sup>b</sup>	2.24 ± 0.20 <sup>b</sup>	0.050
C20:3 n-3	0.01 ± 0.00 <sup>a</sup>	0.09 ± 0.01 <sup>b</sup>	0.11 ± 0.01 <sup>b</sup>	0.013
C20:4 n-6	1.92 ± 0.40	1.82 ± 0.67	2.12 ± 0.87	0.340
C20:5 n-3	0.34 ± 0.11 <sup>a</sup>	0.39 ± 0.17 <sup>a</sup>	0.56 ± 0.12 <sup>b</sup>	0.027
C22:5 n-3	0.37 ± 0.16 <sup>ab</sup>	0.14 ± 0.06 <sup>a</sup>	0.96 ± 0.06 <sup>b</sup>	0.029
SFA	43.92 ± 1.95	46.58 ± 3.57	44.79 ± 3.94	0.302
MUFA	34.16 ± 2.49 <sup>a</sup>	37.63 ± 4.81 <sup>ab</sup>	39.91 ± 2.61 <sup>b</sup>	0.042
PUFA	12.82 ± 2.01 <sup>a</sup>	12.89 ± 2.88 <sup>a</sup>	15.41 ± 2.92 <sup>b</sup>	0.039

<sup>a,b</sup> Different letters within the same row denote significant differences between means.

**Table 3.** Fatty acids ratios of intramuscular fat of lambs under three feeding regimens.

Fatty Acid Ratios	Group I	Group II	Group III	p-Value
∑ 18:1	33.54 ± 2.16 <sup>a</sup>	35.13 ± 2.48 <sup>ab</sup>	37.81 ± 3.08 <sup>b</sup>	0.043
∑ n-6	10.13 ± 1.34	9.98 ± 2.07	10.96 ± 2.85	0.720
∑ n-3	2.65 ± 0.33 <sup>a</sup>	2.90 ± 0.46 <sup>a</sup>	3.86 ± 0.48 <sup>b</sup>	0.014
∑ n-6/∑ n-3	3.82 ± 1.02 <sup>b</sup>	3.43 ± 1.12 <sup>b</sup>	2.87 ± 0.55 <sup>a</sup>	0.035
PUFA/SFA	0.28 ± 0.04 <sup>a</sup>	0.27 ± 0.05 <sup>a</sup>	0.35 ± 0.15 <sup>b</sup>	0.039
Desirable FAs	62.28 ± 2.02 <sup>a</sup>	68.24 ± 3.21 <sup>b</sup>	74.31 ± 3.53 <sup>c</sup>	<0.001
Atherogenic index (AI)	0.75 ± 0.08 <sup>b</sup>	0.79 ± 0.16 <sup>b</sup>	0.61 ± 0.10 <sup>a</sup>	0.027
Desaturase C18 index	0.67 ± 0.12	0.65 ± 0.20	0.66 ± 0.07	0.881

<sup>a-c</sup> Different letters within the same row denote significant differences between means.

The percentage of fatty acids considered beneficial for human health was significantly higher in the meat from pasture-fed lambs than those kept indoors ( $p < 0.001$ ). Specifically, lambs fed on *L. corniculatus* pasture had a greater proportion of these desirable fatty acids compared to those supplemented with white clover (Table 3), primarily due to the higher total PUFA content in Group III meat (Table 2). Furthermore, the atherogenic index (AI) was lowest in lambs fed with *L. corniculatus* ( $p = 0.027$ ), with no significant differences observed between the other two groups. In contrast, the feeding regime did not significantly affect the desaturase C18 index ( $p = 0.881$ ).

### 3.2. Colour Characteristics and pH Value

The pH value of the meat 24 h post-slaughter did not significantly differ between the groups (Table 4). However, notable variations in meat colour were observed (Table 4). Group I exhibited a lighter meat colour (higher  $L^*$  values) compared to Groups II and III ( $p = 0.011$ ). The redness ( $a^*$ ) was significantly higher in the meat from lambs kept indoors (Group I) compared to those supplemented with white clover (Group II), with no significant differences noted for this parameter between Group I and Group III ( $p = 0.041$ ). The degree of yellowness ( $b^*$ ), Hue angle (H), and colour saturation ( $C^*$ ) values were not significantly influenced by the supplemented pasture feeding (Table 4).

**Table 4.** pH value and colour parameters of *longissimus lumborum et thoracis* of lambs under three feeding regimens.

Parameters	Group I	Group II	Group III	p-value
pH 24	5.48 ± 0.52	5.51 ± 0.37	5.54 ± 0.16	0.168
L*	42.16 ± 2.53 <sup>b</sup>	40.05 ± 2.24 <sup>a</sup>	40.96 ± 1.92 <sup>a</sup>	0.011
a*	19.32 ± 1.98 <sup>b</sup>	18.37 ± 1.50 <sup>a</sup>	18.87 ± 1.55 <sup>ab</sup>	0.041
b*	6.67 ± 2.1	6.31 ± 1.91	6.56 ± 0.94	0.098
H	19.22 ± 1.71	18.62 ± 1.62	19.10 ± 1.38	0.277
C*	20.48 ± 2.21	19.40 ± 3.66	19.99 ± 1.75	0.107

<sup>a,b</sup> Different letters within the same row denote significant differences between means.

### 3.3. Mineral Composition

Table 5 presents the mineral content in the *longissimus lumborum et thoracis* muscles of the studied lambs. The analysis indicated that potassium and sodium were the most abundant minerals, followed by magnesium and calcium. Significant variations in calcium, iron, potassium, and selenium content were observed across the different diet groups. Group I had lower calcium and selenium levels than Groups II and III ( $p = 0.007$  and  $p = 0.015$ , respectively).

**Table 5.** Mineral composition of *longissimus lumborum et thoracis* of lambs under three feeding regimens.

Mineral (ppm)	Group I	Group II	Group III	p-Value
Na	1063.72 ± 45.42	969.39 ± 66.08	1000.5 ± 53.27	0.677
Ca	34.25 ± 2.95 <sup>a</sup>	41.00 ± 4.28 <sup>b</sup>	41.60 ± 6.89 <sup>b</sup>	0.007
Mg	229.00 ± 13.33	225.50 ± 9.18	238.60 ± 15.08	0.141
Fe	14.00 ± 0.71 <sup>a</sup>	15.40 ± 1.17 <sup>a</sup>	18.20 ± 0.98 <sup>b</sup>	0.038
Zn	18.25 ± 0.83	19.60 ± 2.06	21.00 ± 1.67	0.277
K	3912.36 ± 199.02 <sup>b</sup>	3521.96 ± 153.20 <sup>a</sup>	3433.51 ± 111.23 <sup>a</sup>	0.038
Se	0.09 ± 0.01 <sup>a</sup>	0.19 ± 0.02 <sup>b</sup>	0.17 ± 0.01 <sup>b</sup>	0.015

<sup>a,b</sup> Different letters within the same row denote significant differences between means.

Additionally, including *L. corniculatus* in the lambs' diet significantly increased iron levels compared to the other two groups ( $p = 0.038$ ). Interestingly, lambs on the supplemented pasture diet exhibited significantly lower potassium levels ( $p = 0.038$ ). As shown in Table 5, all three lamb groups had similar sodium, magnesium, and zinc levels in the analysed meat samples ( $p > 0.05$ ).

## 4. Discussion

### 4.1. Fatty Acids Composition

Given the significant potential to modulate the fatty acid (FA) composition of meat, diet-based strategies have been extensively studied for their ability to enhance the FA profile of lamb meat [13]. Forage degradation in the rumen, a complex process involving numerous microorganisms, is crucial in synthesising long-chain n-3 fatty acids through enzymatic activity [35]. Studies have shown that sheep grazing on pasture exhibit greater microbial diversity than those fed hay and concentrate [36]. Diets rich in phenolic compounds have been demonstrated to increase the content of total n-3 and n-6 FAs while reducing the saturated fatty acid (SFA) content in meat [37,38]. For example, lambs fed silage mixtures containing red clover and sainfoin had higher n-3 content in their meat [39]. Furthermore, it is known that fresh and ensiled herbage results in a more favourable FA profile compared to dry herbage [40]. Fattening lambs exclusively on pasture rather than with concentrate supplementation doubled the total n-3 content and reduced the n-6/n-3 ratio due to the high  $\alpha$ -linolenic acid content in the grass, a precursor for n-3 fatty acids [41]. However, despite the benefits of pasture feeding on the FA profile of lamb meat, keeping animals on pasture is not always feasible, leading to the common practice of concentrate

supplementation. Previous research indicates that this practice can erode some advantages of pure pasture-fed lamb, particularly in increasing monounsaturated fatty acid (MUFA) content and decreasing n-3 content, especially with higher concentrate inclusion in the diet. This effect is attributed to cereal grains' high linoleic acid content, which increases the n-6/n-3 ratio [42].

In this study, pasture feeding with concentrate supplementation improved the FA profile and health indices of lamb meat compared to indoor feeding (Tables 2 and 3). These findings are consistent with previous research indicating that supplemented pasture feeding results in higher n-3 content [41]. McNicol et al. [43] have found a decrease in total SFA, as well as palmitic (C16:0) and stearic (C18:0) acids in the *longissimus dorsi* muscle ( $p < 0.05$ ) when lambs are fed a supplemented grazing system. Additionally, Ponnampalam et al. [44] report that grain-based diets produce higher levels of palmitic (C16:0) and stearic (C18:0) acids compared to pasture-based diets. However, contrary to these studies, our findings did not show a difference in SFA content between groups (Table 2). This discrepancy might be due to factors such as the duration of feeding, animal breed, age at slaughter, and the phenolic content of the pasture [45]. Additionally, grass-fed ruminants are often thought to have higher stearic acid (C18:0) levels than grain-fed animals [46–48], but in this study, this was confirmed only for lambs fed on supplemented *L. corniculatus* pasture (Table 2).

Research has shown that plant secondary metabolites like polyphenol oxidase, flavonoids, tannins, essential oils, and saponins contribute to a better FA profile through higher polyunsaturated fatty acid (PUFA) accretion [38,39]. The total n-3 fatty acids in lamb meat, which provide health benefits like reducing cardiovascular disease and inflammatory risks [49], vary significantly with diet [50]. Legume-rich pastures can produce meat richer in  $\alpha$ -linolenic acid (C18:3 n-3) due to faster rumen outflow rates and less advanced ruminal biohydrogenation [37]. Previous research has shown that lambs on forage and grass diets have higher  $\alpha$ -linolenic acid (C18:3 n-3) levels in the *longissimus dorsi* muscles than those on concentrate diets [43]. This is mainly due to the high C18:3 n-3 content in the grass, synthesised in plant chloroplasts, and influenced by factors such as season, species, location, and environment. McNicol et al. [43] also have found significant increases in other n-3 fatty acids like eicosapentaenoic (C20:5 n-3), docosapentaenoic (C22:5 n-3), and docosahexaenoic (C22:6 n-3) acids in pastured lambs. However, Fisher et al. [50] and Demirel et al. [51] report no significant effects of pasture feeding on these long-chain n-3 fatty acids in lamb meat. In this research, the total n-3 content was highest in lambs fed *L. corniculatus*, with no significant difference between the other two groups (Table 3). This variation in the effect of different forages on meat FA composition was also confirmed by McNicol et al. [43], comparing different forage swards. The authors found that lambs grazing perennial ryegrass (*Lolium perenne*) had a lower n-6/n-3 ratio and a more favourable FA profile compared to those finished on red clover (*Trifolium pratense*) or lucerne (*Medicago sativa*). Additionally, they reported that linoleic acid (C18:2 n-6) levels were highest in lamb's *longissimus dorsi* muscle fed indoors with concentrate and lowest in those on a pure grass diet. However, in our study, the combination of pasture and concentrate did not significantly affect linoleic acid levels (Table 3).

All n-6/n-3 ratios established in this trial exceeded the recommended values (<5) concerning human health [52]. This large n-6/n-3 ratio, which represents nutritionally and functionally significant meat quality, can be influenced by feed, ranging from 0.6 to 49.6 [53]. The n-6/n-3 ratio was lower in lambs fed with *L. corniculatus*, consistent with McNicol et al. [43]. Studies suggest that the n-6/n-3 ratio in phospholipids can distinguish between grass-fed and grain-fed lambs [54]. Furthermore, as red meat is a primary source of SFAs, nutritionists recommend a minimum PUFA/SFA ratio of 0.4 [55]. In this study, the PUFA/SFA ratio increased in lambs fed *L. corniculatus* (Table 3) but remained below the recommended value ( $0.35 \pm 0.15$ ).

Research shows that feeding systems significantly affect the atherogenic (AI) and desaturase C18 indices, with stall-fed lambs showing higher values than pasture-fed lambs [56]. Our findings suggest that an *L. corniculatus*-supplemented feeding regime

significantly lowers the AI index compared to *T. repens* or concentrate feeding (Table 3). The AI values reported here align with the general range for meat fats of 0.5 to 1. Lower AI is favourable for human health, as it indicates a decrease in undesirable FAs. The higher desaturase C18 index suggests higher  $\Delta$ -9 desaturase activity, leading to more C18:1 production [56]. Although indoor lambs (Group I) had lower C18:1 n-9 and C18:1 n-11 content, no significant differences in the desaturase C18 index were observed between groups in this study (Table 3).

#### 4.2. Colour Characteristics and pH Value

Understanding the influence of diet on meat colour and quality is crucial, as it significantly affects consumer purchasing decisions [57]. Consumers tend to reject meat that appears excessively dark or discoloured [58]. The colour of fresh meat is influenced by myoglobin content, its chemical state, muscle tissue structure, and pH [13,59]. Variations in these characteristics can be caused by factors such as the animal's age, diet, and preslaughter stress [60]. For instance, light-suckling lambs exhibit lighter meat colours due to their lower myoglobin levels [61].

The influence of different lamb feeding systems on ultimate muscle pH has yielded inconsistent results across various studies, indicating a need for further research. For instance, a study by Ripoll et al. [58] found that lambs finished on pasture had lower pH 24 values compared to those finished on grain-based feedlot diets. Similarly, other studies indicated that lambs fed a commercial diet exhibited lower ultimate pH values than those on grazing systems [62,63]. However, some studies reported no significant differences in meat pH among different feeding systems [58,64]. Importantly, in this research, the different feeding regimes did not result in significantly different pH values of the *longissimus thoracis et lumborum* (Table 4), highlighting the need for further investigation.

Research generally suggests that meat from pasture-fed animals tends to be darker compared to those on concentrate-based diets [3,62,65]. The highest lightness value ( $L^*$ ) of the *longissimus thoracis et lumborum* muscle was observed in lambs fed indoors with dietary concentrate supplementation, with no significant differences between the other two groups (Table 4). Additionally, pasture-fed animals often exhibit a higher ultimate pH, contributing to darker meat colour [66], likely due to reduced glycogen reserves at slaughter or heightened sensitivity to preslaughter stress [60]. Priolo et al. [62] noted that lambs fed concentrates had lighter meat than those fed grass, partly due to differences in ultimate pH; grass-fed lambs had a pH of 5.62, while concentrate-fed lambs had a pH of 5.57. Interestingly, this study found no significant differences in the pH 24 values of the *longissimus thoracis et lumborum* across groups (Table 4), despite the general association between higher pH and darker meat. No significant differences in  $L^*$  values were found between lambs fed on two pasture systems (Table 4), consistent with De Brito et al.'s [9] findings that different forage diets do not usually affect meat colour. Variability in grazing conditions and lamb growth rates in pasture-based diets may contribute to these differences in meat lightness from grass-fed animals [2].

Meat redness ( $a^*$ ) is primarily influenced by the amount of heme, myoglobin states, and marbling [67]. In studies by Cerdeño et al. [68] and Aguayo-Ulloa et al. [19], the  $a^*$  value did not depend on the lamb production system. However, in this research, supplemented pasture systems significantly affected the  $a^*$  value of the *longissimus thoracis et lumborum* muscle, particularly in animals fed white clover (Group II), which had higher values than Group I (Table 4). This difference could also be due to varying physical activity levels, significantly impacting myoglobin [13].

Pasture and forage diets are often linked with increased meat yellowness due to the diet's carotenoids, flavonoids, and tocopherols [3]. However, in this study, the yellowness ( $b^*$ ) value of the *longissimus thoracis et lumborum* did not differ significantly between groups (Table 4), contrary to most of the literature suggesting an increase with pasture intake [19,28,69]. Nonetheless, these findings align with Wang et al. [2]. Priolo et al. [3] found that higher Hue angle and  $b^*$  values in lambs indicate a more brown colour, associated

with higher metmyoglobin concentrations and lower oxidative stability. Additionally, this research found no influence of diet on the Hue angle (H) and chroma value (C\*) of meat (Table 4).

#### 4.3. Mineral Composition

Lamb and sheep meat are valuable sources of essential minerals like iron, zinc, and selenium [70], with 100 g of lamb meat providing 10–20% of the recommended daily intake of these minerals [42]. Minson [71] provided an extensive review of mineral composition in forages, revealing that legumes typically contain higher levels of Ca, P, Mg, Cu, Zn, and Co, but lower levels of Na and Mn compared to grasses. Previous studies on the mineral composition of lamb meat have identified potassium as the most abundant macroelement, iron and zinc as the most prevalent microelements, and selenium as the least abundant [72,73]. These findings are consistent with the current research results (Table 5).

Meat is a significant source of total and heme iron, primarily comprising myoglobin and haemoglobin, which are crucial for respiration and tissue oxygenation [74,75]. In this study, the highest iron content was found in lambs fed with *L. corniculatus* supplementation (Group III), with no significant differences observed among the other groups (Table 5). The iron levels in this study (14.00 to 18.20 ppm) were lower than those reported by Belhaj et al. [73] for domestic female sheep breeds in Moroccan pastures (26.0 to 29.3 ppm), but higher than those found by Van Heerden et al. [76] in South Africa (9.9 ppm in fresh loin). The iron levels in this experiment were similar to those reported by Pinheiro et al. [77] for lambs raised on *Cynodon dactylon* pasture in Brazil and Kasap et al. [72] for Croatian sheep breeds. Factors such as gender, age, muscle type, rearing location, feeding practices, and processing methods are suggested to influence iron content in lamb meat [78]. Additionally, variations in soil iron levels, affected by soil alkalinity and bioavailability factors, likely contribute to these differences [79].

Selenium is an essential trace element for humans [80], and its concentration in meat is influenced by environmental conditions, animal age, rearing practices, and geographical origin [81]. Mikkelsen et al. [82] reported that plant species, selenium forms, and soil characteristics (pH, texture, organic matter content) affect selenium uptake by plants. Cabrera et al. [75] indicate that the meat of pasture-reared animals is rich in selenium, consistent with this study's findings where selenium levels were significantly higher in pasture-fed lambs compared to controls (Table 5). However, the contents of Se can vary depending on the quality of the pasture [80]. The selenium levels in this study align with those reported by Belhaj et al. [73] and were higher than values reported by Williamson et al. [83] for lamb meat in Denmark, the UK, and Australia (0.06, 0.07, and 0.1 ppm, respectively), but lower than in the USA (3.0 ppm).

Zinc deficiency can lead to anaemia, fatigue, poor growth, and impaired cognitive function [84]. In this study, the feeding system did not affect zinc levels in lamb meat (Table 5). Our results were similar to those found by Kasap et al. [72], but lower than those reported by Belhaj et al. [73], likely due to differences in soil and grass zinc levels on pastures [75]. Pannier et al. [78] also found no significant differences in zinc content in the *semimembranosus* muscle across different diets, though they reported diet effects on zinc levels in the *longissimus thoracis et lumborum* ( $p < 0.001$ ).

## 5. Conclusions

This study demonstrates the significant impact of a supplemented pasture diet on the fatty acid composition, colour, pH, and mineral content of lamb meat. Enhancing the fatty acid profile of intramuscular fat through pasture feeding can improve the health perception of lamb meat by increasing n-3 fatty acids and desirable fatty acids, while reducing the n-6/n-3 ratio and atherogenic index, particularly in lambs finished on an *L. corniculatus* diet. However, this study also notes some potential drawbacks of forage-supplemented feeding on meat colour. Specifically, it was found that the lightness and redness of the analysed muscles were reduced, which may affect the visual appeal of the meat. Conversely,

supplemented pasture feeding improves the meat's mineral profile, with higher levels of calcium and selenium compared to indoor-fed lambs and increased iron content in meat from lambs fed *L. corniculatus*.

In summary, while concentrate supplementation does offer some benefits to meat quality, it falls short of the advantages of supplemented pasture feeding. Pasture-based systems, especially those incorporating *L. corniculatus*, not only enhance the nutritional quality and overall value of lamb meat but also potentially contribute to environmental sustainability. By providing nutritious meat with a lower environmental impact, these systems offer a win–win situation for both consumers and the environment. By addressing these aspects, the research contributes valuable insights that can inform future agricultural practices, dietary recommendations, and environmental policies aimed at promoting sustainable and nutritious food systems. However, the lack of replication at the treatment level is a limitation of this study, which could potentially impact the robustness of the results. Therefore, further research is crucial to address these challenges and validate these findings.

**Author Contributions:** Conceptualisation, N.S., D.R.-M. and B.C.; methodology, N.S., N.M., V.C.P. and I.Ć.; writing—original draft preparation, N.S., D.R.-M., N.M. and M.L.; writing—review and editing, N.S., B.C., V.C.P. and M.L.; investigation, D.R.-M., N.M., I.Ć. and M.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, on the basis of the agreement on the realization and financing of scientific research work of SRO No. 451-03-66/2024-03/200022.

**Institutional Review Board Statement:** This trial was reviewed and approved on 23.07.2020. by the Ethics Commission of the Institute for Animal Husbandry, Belgrade (decision number 323-07-07552/2020-05). The experiment complied with the principles of the Serbian Law 41/2009 concerning animal welfare and Rulebook 39/10 for the handling and protection of animals used for experimental purposes, as well as the EU Council Directive 98/58/EC concerning the protection of farmed animals and Directive 2010/63/EU on the protection of animals used for scientific purposes.

**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** The authors declare no conflicts of interest.

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