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Source / Izvornik: **Agriculture, 2024, 14**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

https://doi.org/10.3390/agriculture14091550

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:204:977177

agriculture

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Dietary Effects of Black-Oat-Rich Polyphenols on Production Traits, Metabolic Profile, Antioxidative Status, and Carcass Quality of Fattening Lambs

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Abstract: The study aimed to establish the dietary effects of black oat rich in polyphenols on the production traits, metabolic profile, antioxidant status, and carcass quality of fattening lambs, after weaning. In the BO group, in the feed mixture, common oats replaced the black oat compared to the CO group. The research comprehensively investigated production indicators, blood metabolic profile, antioxidant status, and lamb carcass quality. No significant differences were found in the fattening or slaughter characteristics of lamb carcasses, except for lower pH_1 values in BO lamb carcasses. Significant increases in RBC, HCT, and MCV levels as well as TP, ALB, and GLOB concentrations and GPx and SOD activities in the blood of BO lambs were found. The glucose and EOS content as well as the activity of the enzymes ALT and ALP were significantly lower in the blood of the BO group than in the CO group. In the liver, the DPPH activity was significantly higher in the BO lambs compared to the CO lambs. The observed changes in glucose, protein metabolism, and antioxidant status in the blood and tissues of lambs indicate that the use of polyphenol-rich black oats in the diet of lambs under stress conditions is justified.

Keywords: oat; black oat; production traits; metabolic profile; antioxidative status

Citation: Antunović, Z.; Klir Šalavardić, Ž.; Mioč, B.; Steiner, Z.; Đidara, M.; Sičaja, V.; Pavić, V.; Mihajlović, L.; Jakobek, L.; Novoselec, J. Dietary Effects of Black-Oat-Rich Polyphenols on Production Traits, Metabolic Profile, Antioxidative Status, and Carcass Quality of Fattening Lambs. *Agriculture* **2024**, *14*, 1550. https://doi.org/10.3390/agriculture14091550

Academic Editors: Mihaela Saracila, Arabela Elena Untea and Petru Alexandru Vlaicu

Received: 2 August 2024
Revised: 4 September 2024
Accepted: 5 September 2024
Published: 7 September 2024

1. Introduction

Oat grain is commonly used in ruminant rations, typically making up to 20% of the feed mixture [1]. Oats are well known for having a distinctive protein composition and high nutritious content. Oat proteins are mostly composed of globulins as opposed to other grain proteins; as a result, their amino acid profile is more balanced [2]. Oats are regarded as a highly valuable grain because of their high fat content and unsaturated fatty acids, which contribute to their high energy and nutritional value [3]. Due to the ban on the use of antibiotics as growth promoters in animal feed, various biostimulants and other feed additives are increasingly being used to increase resistance and have a positive effect on animal immunity. Numerous colored feedstuffs are becoming increasingly important, including coloured cereals used in animal meals. The reason for their use lies in the richness of polyphenols, which in oats are mainly phenolic acids [4], as quality components that contribute to the above. Dietary interventions with polyphenols may alter the gut microbiome response and attenuate the weaning stress related to inflammation. Further, polyphenols

elicit health-favored effects through ameliorating inflammatory processes to improve digestibility and thereby exert a protective effect on animal production [5]. The health effects of the use of oats in human and animal nutrition are well known, given the richness of oats in various biological components [6–8]. These are primarily antioxidants, proteins, lipids, vitamins, minerals, soluble fiber-β-D-glucan (but also cellulose and arabinoxylan), and especially avenanthramides, a unique group of N-cinnamoyl anthranilic acid derivatives found in oats but not in other cereals [9–12]. Recent research has also demonstrated the anti-inflammatory, antiproliferative, and antipruritic properties of oat polyphenols, which may offer extra defense against colon cancer, coronary heart disease, and skin irritation in both humans and animals [13–15]. There are numerous varieties/genotypes of oats, of which the black varieties, the so-called black oats, stand out [16,17]. In the studies of Varga et al. [17], it was found that the DPPH content was increased in black-hulled oats, indicating an increased antioxidant activity associated with a higher content of phenolic compounds in black-hulled oats. According to Fontanella et al. [18], black oats can only be grown as a forage crop or as a forage crop and cereal. There are considerably more studies in the literature in which black oats are used as pasture fodder or as a component of silage, while the available literature contains very few studies on the use of black oat grains in animal nutrition. Most of these studies were carried out in Central and North America (Mexico, Brazil) with ruminants on pasture [19–21] or as a component of silage [20,22]. The aforementioned studies emphasize the positive effects of black oats in animal feed on the production characteristics and profitability of this production. When analyzing the available literature, two papers were found showing the use of black oat grains together with other cereal grains in animal feeding, in fattening lambs, when satisfactory production and slaughter indicators and economic efficiency of production were achieved [23,24].

Sheep are subjected to a variety of physiological and environmental stresses that affect their welfare, reduce their production, and increase the risk of disease [25]. The lamb experiences extreme stress during weaning. According to Lynch et al. [26], weaning is the total physical separation of mother and child as well as the nutritional switch from milk to solid foods. According to Freitas-de-Melo et al. [27], artificial weaning is regarded as one of the most stressful events in farm animals' lives. As such, it is critical to consider the elements influencing those reactions in order to create effective weaning procedures and enhance the welfare of the sheep. The level of shock manifested by reduction in post-weaning growth rate may vary depending on weaning age and weight, the intake of solid feed before weaning as well as health status of the lamb [25]. Lambs may also experience nutritional, social, and emotional changes that result in a significant stress reaction [28]. According to studies by Damián et al. [29] and Freitas-de-Melo et al. [30], abrupt weaning is linked to reduced weight gain, increased alertness and mobility, and decreased resting and feeding behavior in ewes and lambs. In the Republic of Croatia, the normal time for natural weaning of lambs is around 4 months. Therefore, earlier weaning can cause various problems/shock in lambs. Therefore, the time of weaning and quality preparation of lambs before weaning, especially regarding feeding, are very important [31,32]. It is critical to figure out how to reduce the issues listed above that arise from lambs' weaning stress. Feeding the lambs premium feed, preferably with fodder rich in polyphenols or polyphenols extracted from certain plants that offer many health benefits, is one method. In a study using weaned lambs, for instance, Xu et al. [33] discovered that tea polyphenols in the lambs' diets had antioxidant and anti-inflammatory properties comparable to those of antibiotics.

To the best of our knowledge there is no study carried out on lambs fed black oats in their diets affecting the production traits and blood parameters. Finding out how black oat polyphenols affected the metabolic profile, antioxidative status, production traits, and carcass quality of fattening lambs was the study's main goal.

2. Materials and Methods

The European Union Directive 2010/63/EU, the Animal Protection Act (NN 133/06, NN 37/13, and NN 1. kg125/13), the Act on the Protection of Animals Used for Scientific Purposes (NN 55/13), and other relevant legal acts on the welfare of farm animals were all followed in this research. Consequently, the Bioethical Committee for Research on Animals of the Faculty of Agrobiotechnical Science Osijek (22-03, 17 March 2022) accepted the protocol of the animal study.

2.1. Experiment Design and Body Weight Analysis

The study was carried out on 20 Merinoladschaf lambs aged 70 days after weaning on the Sičaja family farm in Gašinci (Osijek-Baranja County, Croatia; 45°2000500 N, 18°1805900 E), during the year 2022. The selected lambs were fed a feed mixture and meadow hay (40:60) as well as salt and water ad libitum. In addition, an acclimatization phase was carried out to accustom the lambs to the new feed, which lasted 7 days. The selected lambs were in the same sex ratio (50 M: 50 F), body weight, age, and in good physical condition and health. The lambs were divided into 2 groups of 10 lambs each. Each group was housed and ate together in an enclosure (5 m × 4 m). The study lasted 30 days, during which body weight was determined at the beginning and end of the study by weighing (Kern EOS 150K50XL animal scales (Kern & Sohn, Balingen, Germany)). A qualified technician evaluated the body condition score (BCS), which was measured using Russel's 5-point scale [34] (1 = thin to 5 = obese).

2.2. Analyses of Feed Mixture and Hay

As per the National Research Council [35], the feed mixtures were provided to the lambs based on their specific needs, with an expected weight of approximately 32 kg. Using a cutting mill (Microtron MB 550; Kinematica, Luzern, Switzerland) to grind the feed mixture and meadow hay to a particle size of 1 mm, samples were taken, dried at 60 °C, and their chemical composition was examined (Table 1). The standard methods of the AOAC [36] were used to analyze the basic chemical composition of the feed. Methods for the determination of proteins, ether extract, dietary fiber, and ash of feeds were described in the work of Antunović et al. [37]. The crude protein content was estimated on the basis of the nitrogen content using the Kjeldahl method. The concentrations of the ether extracts were analyzed using the extraction system B-811 (Buchy, Flawil, Switzerland). The crude fiber content was determined according to the Weende method and the NEM according to INRAE-CIRAD-AFZ [38]. Finally, the crude ash concentration was determined by burning the feed samples at 550 °C for 6 h. The extraction and determination of polyphenols is presented in the work of Jakobek et al. [39]. Samples were weighed and 0.2 g was set in a tube and 1.5 mL of 80% (v/v) methanol was added in water. Then, samples were vortexed and extracted for 15 min with an ultrasonic water bath (RK 100, Bandelin, Berlin, Germany). Afterwards, samples were centrifuged for 10 min at 6739× g (Eppendorf, Hamburg, Germany). The extract was transferred into a separate plastic tube. Total polyphenols were determined by using the Shimadzu UV-1280 spectrophotometer (Shimadzu Europe GmbH, Duisburg, Germany). Total polyphenols were expressed as mg gallic acid equivalents (GAE)/kg of sample weight. Data are presented as the mean of three parallels.

Table 1. Ingredients and chemical composition of the feed mixture and meadow hay for feeding lambs.

Ingredient (%)	Group		Meadow Hay	Black Oat
	CO	BO		
Ingredients composition				
Corn	40.00	40.00		
Oat	15.00 *	-		
Black oat	-	15.00		
Barley	21.30	21.30		
Soybean meal	8.00	8.00		
Soybean toasted	12.00	12.00		
Limestone	0.30	0.30		
Cattle salt	0.40	0.40		
Mineral premix **	3.00	3.00		
Chemical composition (g/kg DM)				
Dry matter	950.00	945.00	946.70	950.20
Crude proteins	155.00	152.90	102.70	73.60
Ether extract	48.30	48.60	7.00	48.70
Crude fibre	22.30	36.90	353.70	101.30
Ash	66.90	60.40	51.50	28.80
NEM, MJ/kg	8.00	7.00	4.50	-
Polyphenols (total; mg gallic acid equivalents (GAE)/kg)	1631.15	2234.63	4730.30	1538.46

CO—control oat, BO—black oat; NEM-net energy for meat production; * content of total polyphenol in oat is 1053.58 mg/kg. ** Mineral-vitamin premix for lambs: 8% Ca, 5% P, 9.5% Na, 2.00% Mg, 400,000 IU vitamin A, 40,000 IU vitamin D, 500 mg vitamin E, 4000 mg Zn, 2000 mg Mn, 60 mg I, 10 mg Co, 50 mg Se.

2.3. Blood Sampling and Analysis

Blood samples were taken from the lambs at the start and end of the study (days 1 and 30). Sterile vacuum tubes (Vacutube®, LT Burnik, Vodice, Slovenia) were used for blood sampling from the jugular vein. The hematological indicators (leukocytes—WBC, erythrocytes—RBC, hemoglobin content—HGB, HCT—hematocrit, mean corpuscular volume—MCV, mean corpuscular hemoglobin—MCH, mean corpuscular hemoglobin concentration-MCHC and platelet count—PLT) were determined on the Sysmex pocH 100 iV hematology 3-Diff analyzer (Sysmex Europe GmbH, Hamburg, Germany). Blood smears stained with the Pappenheim procedure and fixed in air were used to calculate the relative proportion of each kind of leukocyte. An Olympus BX 51® microscope (Olympus, Tokyo, Japan) was used to differentiate the white blood count. Following blood collection, lamb serum was separated using centrifugation for 10 min at 1609.92× *g*. An Olympus AU640 analyzer (Olympus, Tokyo, Japan) was used for analysis. Mineral concentrations (calcium—Ca; inorganic phosphorus—P; magnesium—Mg), biochemical parameters (glucose, urea, total proteins, cholesterol, albumin, globulin, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and enzyme activities (glutathione peroxidase-GPx, superoxide dismutase-SOD, alanine aminotransferase—ALT; aspartate aminotransferase—AST; gamma-glutamyl transferase—GGT and alkaline phosphatase—ALP) were determined in serum using Olympus System Reagents (Olympus Diagnostica GmbH, Lismeehan, Ireland). The globulin content was determined as a subtraction of total protein and albumin.

2.4. Analyses of Carcass Measures and Meat Quality

The lambs were weighed on an automated animal scale, the Kern EOS 150K50XL (Kern und Sohn, Balingen, Germany), prior to slaughter. On the Croatian market, lamb is sold as whole carcass with head and kidneys. This is why these parts were not separated from the carcass or weighed separately. After slaughter (classic method of bleeding by

severing the large blood vessels in the neck external jugular vein and common carotid artery) and bleeding of 10 lambs, the skin of the lambs was removed from the carcasses and the organs of the abdominal cavity (forestomach, stomach, spleen, intestines, and liver) and the thoracic cavity (trachea with lungs and heart) were cut off. Weighing was conducted on the skin, lower limbs, internal organs, and carcasses itself. The carcasses were measured using the standard developmental technique (linear measurement) according to Antunović et al. [40]: length (carcass length 1—os pubis to the atlas; length 2—os pubis to the first rib; length 3—os pubis to the last rib); circumference of the carcass at chest; length of the hind legs (tuber calcanei to tubercule ossis ischia); and circumference of the hind legs (the widest part). Evaluation of meat quality samples of lamb meat (musculus semimembranosus) was taken from all lambs immediately after carcass processing. The pH_1 values and the color of the lamb carcass were determined 45 min post-mortem, while pH_2 was determined 24 h post-mortem. The pH of muscle (a central part) was measured with a Mettler Toledo contact pH meter (Mettler Toledo, Columbus, OH, USA). In accordance with the CIE [41] L*a*b* color system standard, the meat's color was measured using a portable Minolta Chroma Metre CR-410 (Minolta Camera Co. Ltd., Osaka, Japan). The formula for calculating dressing percentage was (pre-slaughter weight − carcass weight × 100). Water holding capacity was measured according to the method of Sierra [42].

The hue angle and colorfulness were computed using the formulas:

$$H^* = \tan^{-1} (b^*/a^*) \times (180/\pi) \quad (1)$$

$$C^* = \sqrt{} (a^*2 + b^*2) \quad (2)$$

2.5. Extraction of Antioxidants from Lamb Muscle, Liver, and Kidney Samples to Assess Antioxidative Status

Immediately after slaughter, muscle (musculus semimembranosus), liver, and kidney samples were taken and observable fat was detached. Homogenates were prepared in 0.05 M phosphate buffer (pH 7) using an Ultra Turrax (IKA T18 Basic, Labortechnik, Staufen, Germany) homogenizer (10 w/v) and centrifuged at 12,000× g for 60 min at 4 °C. The equipment used was a centrifuge Hermle Z 326 K (Hermle Labortechnik GmbH, Wehingen, Germany). The extraction was performed in triplicate. The prepared extracts were stored at −80 °C in a Binder UF V 700 Ultra-low temperature freezer (BINDER GmbH, Tuttlingen, Germany) until they were analyzed. The supernatant obtained was used for DPPH radical scavenging activity and TBARS.

2.5.1. Free-Radical-Scavenging Activity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH)

With slight adjustments, the DPPH radical scavenging assay previously reported by Qwele et al. [43] was used to measure the extracts' overall antioxidant activity. A volume of 625 µL of 0.2 mM DPPH prepared in methanol was added to 625 µL of supernatant, which had been diluted with distilled water at a 1:4 ratio. The mixture was vortexed and allowed to stand for 30 min in the dark at room temperature. A tube containing 625 µL methanol and 625 µL DPPH solution was used as a control, while methanol alone served as a blank. To establish a baseline, ascorbic acid (AA) was utilized. We took three duplicate measurements for each. DPPH scavenging activity was calculated using Equation (1), and absorbance was measured at 517 nm using a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA).

$$\text{DPPH activity} = ((Ab + As - Am)/Ab) \times 100 \quad (3)$$

where Ab is the absorbance of the 0.1 mM DPPH radical solution at λ = 517 nm, As is the absorbance of the extracts at λ = 517 nm, and Am is the absorbance of the mixture of tested extracts and DPPH radical at 517 nm.

2.5.2. Thiobarbituric-Acid-Reactive Substances (TBARS)

The TBARS method was used to quantify lipid peroxidation in muscle (musculus semimembranosus), liver, and kidney samples. Slight adjustments were made to the parameters published in Liu et al.'s [44] paper. In brief, a volume of 1.5 mL of 0.1% trichloroacetic acid was added to 300 µL of supernatant and centrifuged at 6000× *g* for 5 min at 4 °C. The equipment used was a centrifuge Hermle Z 326 K (Hermle Labortechnik GmbH, Wehingen, Germany). A volume of 800 µL TBA/TCA solution reagent (0.5% 2-thiobarbituric acid in 20% trichloroacetic acid) was added to 400 µL supernatant, vortexed, and heated to 95 °C for 30 min in a boiling water bath. The mixtures were then cooled on ice for 10 min and centrifuged at 18,000× *g* for 15 min at 4 °C with a centrifuge Hermle Z 326 K (Hermle Labortechnik GmbH, Wehingen, Germany). The same procedure without adding the sample was performed for the blank and the absorbance values were measured (532 nm and 600 nm) using a spectrophotometer (Lambda 25, Perkin Elmer, Waltham, MA, USA). The concentration of malondialdehyde (MDA) was calculated using the molar extinction coefficient of MDA (156,000 M^{-1} cm^{-1}). The results were expressed as mg MDA equivalents per kg tissue sample.

2.6. Statistical Analysis

The normality of data distribution was checked by the Shapiro–Wilk test. The mean values of the obtained results were calculated by MEANS procedures in the statistical program TIBICO Statistica® (version 13.3.0) [45]. Differences between mean values of analyzed results were examined by ANOVA using feeding treatment as a fixed effect.

3. Results

3.1. Production Traits

There were no significant variations in the lambs' production traits based on the feeding, as indicated by Table 2. The analysis of slaughter characteristics of lamb carcasses (Table 3) and its development (Table 4) did not reveal any significant changes. Physical properties of lamb carcass are presented in Table 5 in which a decreased value of pH_1 musculus semimembranosus 45 min post-mortem was determined.

Table 2. Production traits of lambs.

Traits	Measuring Time (Days)	Groups (Mean ± sd)		SEM	*p*-Values
		CO	BO		
Body mass (kg)	1	23.71 ± 1.58	24.75 ± 1.34	0.34	0.13
	30	33.71 ± 2.65	34.00 ± 2.27	0.54	0.80
Daily gain (kg)	1–30	0.333 ± 0.05	0.309 ± 0.05	0.01	0.29
Feed conversion (DM/g gain)	1–30	2.86	2.88		
Feed consumption (g/days)	1–30	0.95	0.89		
BCS (point)	1	3.53 ± 0.27	3.66 ± 0.23	0.06	0.26
	30	3.88 ± 0.23	4.00 ± 0.21	0.05	0.06

sd—standard deviation; SEM—standard error of the mean; BCS—body condition score; DM—dry matter; CO—control oat group, BO—black oat group.

Table 3. Slaughtering characteristics of lamb carcasses.

Indicators	Groups (Mean ± sd)		SEM	*p*-Value
	CO	BO		
Pre-slaughter mass (kg)	33.71 ± 2.65	34.00 ± 2.27	0.54	0.80
Carcass mass (kg)	19.06 ± 1.33	18.47 ± 1.03	0.27	0.28
Entrails mass * (kg)	1.50 ± 0.30	1.45 ± 0.26	0.06	0.71
Skin and legs mass (kg)	4.52 ± 0.40	4.64 ± 0.59	0.11	0.60

Table 3. *Cont.*

Indicators	Groups (Mean ± sd)		SEM	*p*-Value
	CO	BO		
Forestomach and intestines mass (kg)	7.78 ± 0.87	7.09 ± 0.59	0.18	0.06
Carcass dressing (%)	56.73 ± 4.54	54.37 ± 1.50	0.78	0.14

sd—standard deviation; SEM—standard error of the mean; * lungs, trachea, heart, liver; CO—control oat group, BO—black oat group.

Table 4. Development measures of lamb carcasses.

Measures	Groups (Mean ± sd)		SEM	*p*-Value
	CO	BO		
Carcass length 1 (cm)	73.90 ± 3.03	74.40 ± 3.06	0.67	0.72
Carcass length 2 (cm)	51.95 ± 2.59	53.40 ± 3.45	0.68	0.30
Carcass length 3 (cm)	29.00 ± 1.53	29.90 ± 1.70	0.37	0.23
Carcass circumference (cm)	65.85 ± 1.84	66.90 ± 1.58	0.44	0.06
Hind legs length (cm)	30.60 ± 4.01	30.15 ± 2.54	0.73	0.21
Ham circumference (cm)	52.60 ± 1.90	54.05 ±1.89	0.44	0.11

sd—standard deviation; SEM—standard error of the mean; carcass length 1 = (os pubis–atlas); carcass length 2 = (os pubis–first rib); carcass length 3 = (os pubis–last rib); CO—control oat group, BO—black oat group.

Table 5. Physical properties of lamb carcasses.

Indicators	Groups (Mean ± sd)		SEM	*p*-Value
	CO	BO		
pH_1	6.51 ± 0.15	6.37 ± 0.10	0.03	0.03
pH_2	5.69 ± 0.05	5.66 ± 0.05	0.12	0.24
WHC (%)	25.50 ± 5.07	28.23 ± 3.88	1.03	0.19
		Color		
L*	44.32 ± 1.24	43.00 ± 1.82	0.37	0.08
a*	19.03 ± 1.41	19.30 ± 0.80	0.25	0.14
b*	2.47 ± 0.26	2.42 ± 0.27	0.06	0.68
Hue angle	7.44 ± 0.90	7.17 ± 0.88	0.20	0.51
Chroma	19.19 ± 1.40	19.45 ± 0.79	0.25	0.61

sd—standard deviation; SEM—standard error of the mean; CO—control oat group, BO—black oat group; WHC—water holding capacity.

3.2. Blood Parameters and Antioxidative Status

Table 6 displays the hematological indicators in which a significant increase in RBC, HCT, and MCV content, and a decrease in EOS was presented at the end of study in the blood of the BO group. Table 7 presents the biochemical indicators in which significant differences were observed, namely a significant increase in TP, ALB, and GLOB concentrations, but also a significant decrease in glucose concentrations at the end of the study in the blood of the BO groups compared to CO. Enzyme activities are presented in Table 8 when feeding lambs feed mixture with black oats. Significant differences were observed in GPx and SOD activities, but a significant decrease in ALT and ALP enzyme activity was also found at the end of the study in the blood of the BO groups compared to CO.

TBARS content in all tissues in BO was lowered compared to the CO group but not significant (Figure 1). Non-significant changes in the DPPH scavenging activity were found in the muscle and kidney. However, a numerical increase in the DPPH scavenging activity was found in all tissues in BO group. Figure 2 shows that BO lambs had a significantly higher DPPH scavenging activity in the liver compared to CO lambs.

Table 6. Hematological parameters in lambs.

Indicators	Measuring Time (Day)	Groups (Mean ± sd)		SEM	*p*-Values	Ref. Values [46]
		CO	BO			
WBC × 10^9 L	1	10.51 ± 2.74	10.39 ± 1.85	0.51	0.91	5.10–15.90
	30	11.31 ± 3.02	12.12 ± 3.60	0.73	0.59	
RBC × 10^{12} L	1	9.74 ± 1.19	10.50 ± 0.88	0.24	0.14	9.20–13.00
	30	9.17 ± 3.02	10.45 ± 0.63	0.27	0.01	
HGB (g/L)	1	117.70 ± 9.90	120.70 ± 2.62	2.02	0.47	105.00–137.00
	30	121.80 ± 10.41	129.00 ± 9.84	2.35	0.13	
HCT (L/L)	1	0.44 ± 0.11	0.48 ± 0.12	0.03	0.41	0.28–0.47
	30	0.34 ± 0.06	0.40 ± 0.03	0.01	0.003	
MCV (fL)	1	46.09 ± 18.50	46.50 ± 15.24	0.69	0.96	28–41
	30	36.46 ± 0.06	38.09 ± 1.29	0.35	0.02	
MCH (pg)	1	12.22 ± 1.86	11.57 ± 0.62	0.31	0.31	10–13
	30	13.61 ± 3.02	12.41 ± 1.54	0.54	0.28	
MCHC (g/L)	1	279.00 ± 41.70	261.30 ± 43.92	9.54	0.37	332–392
	30	357.90 ± 101.63	327.80 ± 50.85	18.34	0.20	
PLT × 10^9 L	1	680.80 ± 68.87	634.60 ± 155.73	26.74	0.40	426.00–1142.00
	30	610.53 ± 47.09	591.80 ± 129.74	21.35	0.67	
Differential blood smears (%)						
EOS	1	4.50 ± 3.60	5.40 ± 8.46	1.42	0.76	1–8 [47]
	30	2.60 ± 1.65	1.00 ± 0.67	0.33	0.01	
SEG	1	33.30 ± 7.82	29.20 ± 5.79	1.70	0.07	10–50 [47]
	30	26.10 ± 6.74	24.40 ± 11.02	1.99	0.68	
BAC	1	0.20 ± 0.42	0.40 ± 1.71	0.37	0.06	0 [47]
	30	0	0.10 ± 0.32	0.05	0.33	
LYMPH	1	56.60 ± 9.24	64.50 ± 11.96	2.50	0.16	50–75 [47]
	30	69.50 ± 8.44	73.60 ± 10.78	2.16	0.36	
MONO	1	4.80 ± 6.63	0.90 ± 1.20	1.13	0.08	0–4 [47]
	30	1.70 ± 2.00	0.70 ± 0.95	0.36	0.17	
BAS	1	0.59 ± 0.70	0.60 ± 1.08	0.20	0.99	0–1 [47]
	30	0.10 ± 0.32	0.20 ± 0.42	0.08	0.56	

sd—standard deviation; SEM—standard error of the mean; CO—control oat group, BO—black oat group; WBC—white blood cells; RBC—red blood cells; HGB—hemoglobin content; HCT—hematocrit; MCV—mean corpuscular volume; MCH—mean corpuscular hemoglobin; MCHC—mean corpuscular hemoglobin concentration; PLT—platelet count; SEG—segmented neutrophils; LYMPH—lymphocytes; EOS—eosinophils; MONO—monocytes; BAS—basophils; BAC—band cells.

Table 7. Lambs' blood biochemical parameters.

Indicators	Measuring Time (Day)	Groups (Mean ± sd)		SEM	*p*-Values	Ref. Values [46]
		CO	BO			
Ca (mmol/L)	1	2.77 ± 0.16	2.89 ± 0.23	0.05	0.20	2.42–2.92
	30	2.85 ± 0.21	2.68 ± 0.28	0.08	0.12	
P-inorganic (mmol/L)	1	3.04 ± 0.60	3.31 ± 0.52	0.13	0.29	1.88–3.34
	30	3.05 ± 0.35	2.99 ± 0.38	0.10	0.08	
Mg (mmol/L)	1	1.55 ± 0.19	1.61 ± 0.22	0.05	0.52	0.91–1.31
	30	1.52 ± 0.18	1.42 ± 0.14	0.04	0.17	
Glucose (mmol/L)	1	6.62 ± 0.52	6.39 ± 0.58	0.12	0.37	2.70–4.80
	30	6.57 ± 0.34	6.06 ± 0.67	0.13	0.046	

Table 7. *Cont.*

Indicators	Measuring Time (Day)	Groups (Mean ± sd)		SEM	*p*-Values	Ref. Values [46]
		CO	BO			
Urea (mmol/L)	1	6.55 ± 1.07	6.80 ± 1.83	0.33	0.72	5.00–9.10
	30	9.76 ± 1.51	9.67 ± 0.97	0.28	0.89	
Total proteins (g/L)	1	60.80 ± 4.01	59.19 ± 4.32	0.93	0.40	51.00–64.00
	30	58.50 ± 3.19	65.64 ± 4.30	1.16	0.007	
ALB (g/L)	1	30.87 ± 2.47	28.97 ± 2.01	0.54	0.08	30.00–37.00
	30	28.64 ± 4.57	32.32 ± 1.69	0.86	0.03	
GLOB (g/L)	1	29.93 ± 3.63	30.22 ± 3.64	0.79	0.86	19.00–30.00
	30	29.82 ± 3.62	33.33 ± 3.33	0.86	0.04	
CHOL (mmol/L)	1	1.42 ± 0.46	1.63 ± 0.76	0.14	0.46	1.35–1.97 [48]
	30	1.43 ± 0.22	1.29 ± 0.30	0.06	0.26	
HDL (mmol/L)	1	0.73 ± 0.26	0.87 ± 0.19	0.05	0.19	0.68–0.97 [49]
	30	0.83 ± 0.09	0.77 ± 0.17	0.03	0.09	
LDL (mmol/L)	1	0.42 ± 0.26	0.64 ± 0.49	0.09	0.22	0.10–0.50 [49]
	30	0.45 ± 0.14	0.39 ± 0.17	0.03	0.80	
TRIG (mmol/L)	1	0.43 ± 0.10	0.40 ± 0.07	0.02	0.48	
	30	0.33 ± 0.14	0.29 ± 0.04	0.02	0.78	0.00–2.00 [48]
NEFA (mmol/L)	1	0.35 ± 0.47	0.17 ± 0.18	0.08	0.27	<0.4
	30	0.27 ± 0.22	0.26 ± 0.14	0.04	0.88	
BHB (mmol/L)	1	0.29 ± 0.12	0.28 ± 0.13	0.03	0.87	0.2–0.7
	30	0.30 ± 0.10	0.38 ± 0.12	0.03	0.11	

sd—standard deviation; SEM—standard error of the mean; CO—control oat group, BO—black oat group; Ca—calcium; P—inorganic phosphorus; Mg—magnesium; ALB—albumin; GLOB—globulin; CHOL—cholesterol, TRIG—triglycerides, HDL—high-density lipoprotein; LDL—low-density lipoprotein; NEFA—non-esterified fatty acids; BHB—β-hydroxybutyrate.

Table 8. Blood enzyme activity in lambs.

Indicators	Measuring Time (Day)	Groups (Mean ± sd)		SEM	*p*-Values	Ref. Values [46]
		CO	BO			
AST (U/L)	1	107.88 ± 8.84	116.03 ± 11.98	2.48	0.10	83.00–140.00
	30	130.55 ± 16.11	119.27 ± 5.76	2.93	0.052	
ALT (U/L)	1	13.43 ± 3.24	15.29 ± 5.24	0.97	0.35	6.00–20.00 [48]
	30	16.06 ± 2.53	12.55 ± 0.69	0.57	0.0006	
ALP (U/L)	1	441.97 ± 114.50	459.56 ± 98.01	23.28	0.72	300–500 [50]
	30	590.11 ± 59.36	508.66 ± 64.23	16.39	0.009	
GGT (U/L)	1	81.31 ± 10.09	68.58 ± 19.44	3.67	0.08	56.00–110.00
	30	72.49 ± 9.50	70.10 ± 10.30	2.14	0.59	
GPx (U/L)	1	524.02 ± 37.77	524.73 ± 38.98	8.35	0.97	>600 [50]
	30	510.27 ± 36.74	676.73 ±109.68	25.85	0.0005	
SOD (U/mL)	1	0.39 ± 0.10	0.43 ± 0.11	0.04	0.366	0.39–0.67 [50]
	30	0.54 ± 0.08	0.63 ± 0.10	0.03	0.047	

sd—standard deviation; SEM—standard error of the mean; CO—control oat group, BO—black oat group; (LDL), AST—aspartate aminotransferase; ALT—alanine aminotransferase; ALP—alkaline phosphatase; GGT—gamma-glutamyl transferase; GPx—glutathione peroxidase; SOD—superoxide dismutase.

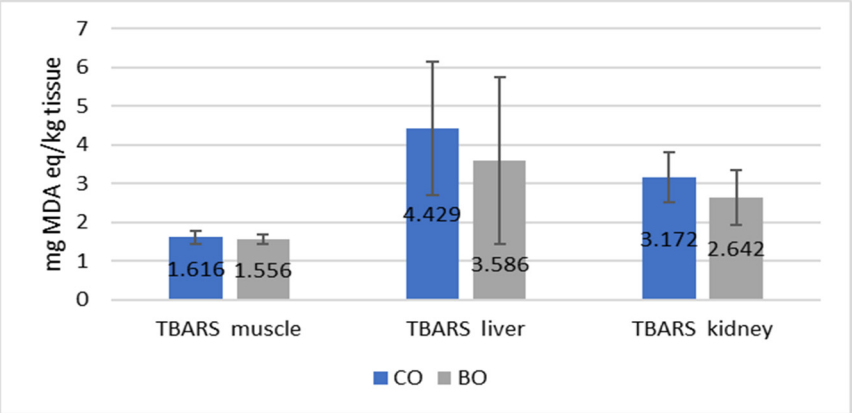

Figure 1. Content of TBARS (reactive thiobarbituric acid substances) in muscle (musculus semimembranosus), liver, and kidney of lambs from the CO (control oat) and BO groups (black oat).

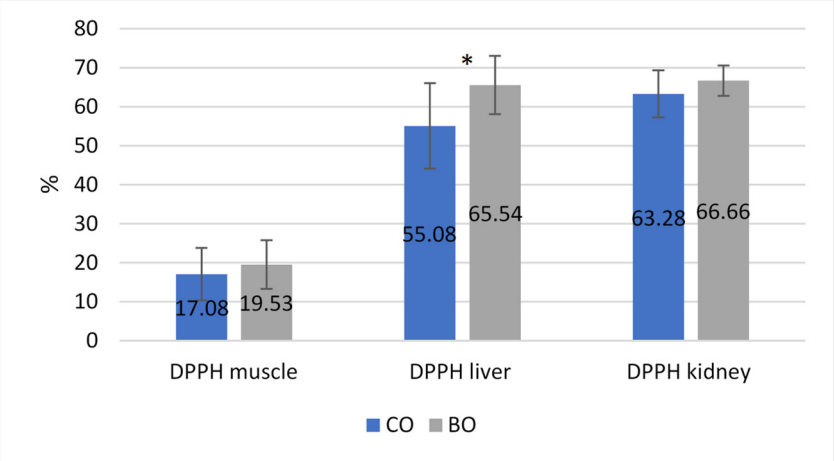

Figure 2. DPPH (2,2-diphenyl-1-picrylhydrazyl radical) scavenging activity in muscle (musculus semimembranosus), liver, and kidney of lambs from CO (control oat) and BO groups (black oat); * *p*-values for DPPH-liver is 0.031.

4. Discussion

4.1. Production Traits

It is well known that weaning is a very stressful period for the ruminants since it is a process in which milk is gradually replaced with concentrates or diets based on grains and forage. It has been confirmed that sheep's and goats' high metabolic demands make them more vulnerable to oxidative stress. The occurrence of oxidative stress in ruminants jeopardizes their productivity and health. One of the main dietary sources of polyphenols is cereal, particularly whole grains [51]. Whole grains contain non-nutritive chemicals called polyphenols, which are mostly flavonoids, lignans, and phenolic acids and found in all structural domains of cereal components. The influence of polyphenols in stress during weaning of young domestic animals (calves, lambs, piglets) improves digestion and absorption of nutrients, improves the function of the intestinal barrier, improves the function of the intestinal microbiota, and thus provides positive effects [52]. In the present study, no significant effect of black oats in the diet was found on the production traits of lambs (daily gain, BCS, conversion, and feed consumption), the slaughter traits of lamb carcasses (weight, individual parts of the carcass and yield, developmental measures, and physical characteristics of carcasses, with the exception of pH1). Bernardes et al. [23,24] obtained satisfactory production and slaughter indicators for lambs when using black oats together with other grains in the meals of fattening lambs. In the study by Tian et al. [53] conducted with goat kids at the age of 90 days (weight 21.38 kg) with the aim of

investigating the influence of anthocyanins from purple corn on traits, meat quality and antioxidant activity of muscles, no influence on the performance of kids (daily gain, feed conversion, feed consumption) was determined. In the study by Orzuna-Orzuna et al. [54], the effects of a polyphenol-containing polyherbal mixture (PM) on growth performance, carcass characteristics, and meat quality in lambs during the fattening period were investigated. The above authors found no changes in final body weight, body condition, carcass characteristics, and meat quality between treatments ($p > 0.05$). Cimmino et al. [55] found that there were no differences in productive traits (daily gain, carcass weight, pH and percentage of dressing) in a study with additional dietary supplementation with polyphenols (3.2 mg/day for 78 days) from the residual water after olive oil extraction in Saanen goat kids. Since consumers are guided by color when choosing fresh meat, the same authors have demonstrated the importance of color for meat quality. The meat of the lambs did not change in color during the current investigation. Similar conclusions were reached in studies in which the addition of polyphenols and flavonoids to the diet had no effect on the color of the meat of lambs [56].

4.2. Blood Parameters and Antioxidative Status

The metabolic profile of blood can be used to diagnose and predict disease and to assess the physiology and welfare of animals [57]. The beneficial effects of polyphenols in animals depend largely on their bioavailability in target tissues [58], cellular distribution, and metabolism after absorption. The analysis of the metabolic profile of the lambs' blood showed a significant increase in the content of hematological indicators (RBC, HCT, and MCV) and a decrease in the content of EOS in the blood of the lambs in the BO group compared to the CO group. All hematological parameters in the blood of lambs showed very little variation overall, with the results being within the reference range for lambs [37,47,59]. In their study, Teng et al. [60] concluded that the positive effect of polyphenols on erythropoiesis could be beneficial both in healthy people and in patients with metabolic diseases. Youdim et al. [61] showed that polyphenols are known to protect against reactive oxygen-species-induced hemolysis by increasing red blood cell integrity in conjunction with inhibition of lipid peroxidation. In the study by Orzuna-Orzuna et al. [54], the effects of a polyphenol-containing polyherbal mixture (PM) on blood metabolite concentrations in lambs during the fattening period was studied. Authors found an increase ($p < 0.05$) in MCHC and lymphocytes and a decrease in SEG in experimental lamb's blood fed the PM diet compared to the CON treatment ($p < 0.05$), which they classify as beneficial for the health status of the lambs. The analysis of biochemical indicators and enzyme activity in the blood revealed significantly higher concentrations of TP, ALB, and GLOB as well as GPx and SOD activities but also a significant decrease in glucose concentrations and ALT and ALP enzyme activity at the end of the study in the blood of the BO groups compared to the CO group. It is also noticeable that there was a decrease in total, high-density lipoprotein and low-density lipoprotein and triglycerides in the blood of the lambs in the BO group compared to the CO group, but without significant differences ($p > 0.05$). The biochemical indicators determined in the blood were within the reference values for lambs [46,49,62]. Higher protein concentration in the lamb's blood of the BO group can be associated with increased amino acid and microbial protein flux in the duodenum of ruminants fed flavonoids [63], while increased globulin levels in serum are an indicator of an improved immune response of the lamb's body [57]. The liver plays a very important role in protein metabolism. Any damage to its cells is reflected in the total serum proteins [64]. Giannenas et al. [65] showed that the addition of a plant additive rich in polyphenolic compounds to the ration of cows reduced blood urea and increased serum globulin and total protein concentrations, improved nutritional status, increased microbial protein synthesis and minimized protein catabolism. When compared to the CO group, the glucose levels in the blood serum of the BO lambs was significantly lower at the last of the research. In a study by Antunović et al. [37], a lower blood glucose concentration was found when red corn in the ration of fattening lambs compared to the blood of lambs

fed yellow corn. Even at low concentrations (1%), oat β-glucans are thought to have a high viscosity, which may contribute to lower plasma glucose and insulin levels as seen in type 2 diabetics by delaying intestinal transit, gastric emptying, and glucose and sterol absorption [66]. The activity of aspartate transferase in blood, gamma-glutamyl transferase, alkaline phosphatases, and cholesterol concentrations are used to diagnose hepatic injury in people and animals, alkaline phosphatases activity and cholesterol concentrations are used to indicate biliary blockage or mild and progressive liver damage [67]. ALT, a particular hepatic enzyme generated following hepatocellular injury, is utilized to measure liver damage instead of GGT. The current study's much lower ALT and ALP at the conclusion imply that neither liver nor muscle damage occurred because their activities were within the reference levels [48]. Antioxidant enzymes such as SOD, GPx, and catalase help to maintain a healthy level of antioxidants within cells [68].

In the present study, significantly higher GPx and SOD activities in the blood, significantly higher DPPH scavenging activity in the liver of BO lambs compared to CO lambs were found. In the study by Tian et al. [53] conducted study with goat kids at the age of 90 days with the aim to research the influence of anthocyanins from purple, determined a significantly higher DPPH scavenging activity and peroxidase levels in muscle (longissimus thoracis et lumborum). In the muscle and kidney samples, no significant changes ($p > 0.05$) were found in the TBARS content and DPPH scavenging activity, although lower TBARS content and higher DPPH scavenging activity were determined in the BO groups in comparison to the CO group, especially in the liver. The changes mentioned indicate a moderate antioxidant activity in the BO group compared to the CO group. It is to be expected that a longer duration of the study would result in a significantly more pronounced positive influence on the antioxidant status of the meat and kidney of lambs in the BO group. Similar results in lambs fed with feed mixture with added red corn were determined by Antunović et al. [37]. Changes in antioxidant status in the kidneys may be associated with the specific uptake or excretion of some tissue metabolites or intracellular metabolism [69]. In recent years, the health effects of whole-grain products have been closely associated with their phenolic compounds and antioxidant effects [51]. Polyphenols not only have strong antioxidant properties [70] but also have bacteriostatic [71], hepatoprotective [72], cholesterol-lowering [73], and immune-boosting [74] effects. In rations rich in polyphenols, an inhibitory effect on the lipid oxidation of meat (reduction of MDA levels) in lamb [75], an increase in the percentage of inhibition of lipid oxidation, an increase in superoxide dismutase activity in the blood of goats [43], and an inhibitory effect on lipid oxidation (reduction of MDA levels) in goat kids [55] were observed in small ruminants. In the current study, a lower pH_1 value was found in the carcasses of BO lambs compared to the CO group. The lamb's pH_2 was within the usual range of 5.5 to 5.8, despite the lamb's pH being similar among treatments [76]. According to Dalle's research [77], meat preservation during storage depends on maintaining an acting bacteriostatic pH below 5.8. Higher pH values, on the other hand, promote the proliferation of proteolytic microbes. The above opens up the possibility of using black oats in other types of ruminants in the future research. This is especially important during the stressful conditions during the weaning period.

5. Conclusions

The observed changes in glucose, protein metabolism in the of lamb's blood and tissues antioxidant status indicate that the use of polyphenol-rich black oats is justified when feeding lambs under stressful conditions, such as in the post-weaning period. In the future, it would be more beneficial to obtain the study with a longer period of feeding lambs with black oats, with its larger amount in the mixture, especially in older sheep, which may possibly improve the antioxidant activity of the meat. Therefore, a polyphenol profile in feed and meat also needs to be carried out in the future.

Author Contributions: Investigation, methodology, writing—original draft preparation conceptualization, visualization Z.A.; investigation, formal analysis, methodology, statistical analyses J.N.;

investigation, visualization, editing Ž.K.Š.; field experiment V.S.; formal analysis, methodology Z.S., M.Đ., V.P., L.M. and L.J.; visualization B.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki by obeying legal provisions determined by the Animal Protection Act (Republic of Croatia Official Gazette No. 133 (2006), No. 37 (2013), and No. 125 (2013)) and approved by the Committee for Animal Welfare of the Faculty of Agrobiotechnical Sciences Osijek (644-01/22-01/01; 22-03 from 17 March 2022).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The research was carried out with the Breeding and Technological Processes in Animal Production (no. 1152) research team at the Faculty of Agrobiotechnical Sciences Osijek.

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

References

1. Lammer, P. *Small Grains for Livestock. A Meta-Analysis*; University of Wisconsin-Platteville: Platteville, WI, USA, 2017.
2. Klose, C.; Schehl, B.D.; Arendt, E.K. Fundamental study on protein changes taking place during malting of oats. *J. Cereal Sci.* **2009**, *49*, 83–91. [CrossRef]
3. Čech, M.; Ivanišová, E.; Hozlár, P.; Tokár, M.; Zagula, G.; Gumul, D.; Kačániová, M.; Sterczyńska, M.; Haščík, P. Nutritional composition, biological activity and technological properties of new Slovakian black oat varieties. *Microbiol. Biotech. Food Sci.* **2021**, *11*, e4238. [CrossRef]
4. Xie, F.; Lei, Y.; Han, X.; Zhao, Y.; Zhang, S. Antioxidant ability of polyphenols from black rice, buckwheat and oats: In vitro and in vivo. *Czech J. Food Sci.* **2020**, *38*, 242–247. [CrossRef]
5. Hussain, T.; Wang, J.; Murtaza, G.; Metwally, E.; Yang, H.; Kalhoro, M.S.; Kalhoro, D.H.; Rahu, B.A.; Tan, B.; Sahito, R.G.A.; et al. The role of polyphenols in regulation of heat shock proteins and gut microbiota in weaning stress. *Oxid. Med. Cell. Longev.* **2021**, *6*, 6676444. [CrossRef] [PubMed]
6. Ivanišová, E.; Čech, M.; Hozlár, P.; Zaguła, G.; Gumul, D.; Grygorieva, O.; Makowska, A.; Kowalczewski, P.Ł. Nutritional, antioxidant and sensory characteristics of bread enriched with whole meal flour from Slovakian black aat varieties. *Appl. Sci.* **2023**, *13*, 4485. [CrossRef]
7. Paudel, D.; Dhungana, B.; Caffe, M.; Krishnan, P. A Review of Health-Beneficial Properties of Oats. *Foods* **2021**, *10*, 2591. [CrossRef] [PubMed]
8. Sur, R.; Nigam, A.; Grote, D.; Liebel, F.; Southall, M.D. Avenanthramides, polyphenols from oats, exhibit anti-inflammatory and anti-itch activity. *Arch. Dermatol. Res.* **2008**, *300*, 569–574. [CrossRef]
9. Tang, Y.; Li, S.; Yan, J.; Peng, Y.; Weng, W.; Yao, X.; Gao, A.; Cheng, J.; Ruan, J.; Xu, B. Bioactive components and health functions of oat. *Food Rev. Int.* **2022**, *39*, 4545–4564. [CrossRef]
10. Singh, R.; De, S.; Belkheir, A. *Avena sativa* (oat) a potential nutraceutical and therapeutic agent: An overview. *Crit. Rev. Food Sci. Nutr.* **2013**, *53*, 126–144. [CrossRef]
11. Alemayehu, G.F.; Forsido, S.F.; Tola, Y.B.; Teshager, M.A.; Assegie, A.A.; Amare, E. Proximate, mineral and anti-nutrient compositions of oat grains (*Avena sativa*) cultivated in Ethiopia: Implications for nutrition and mineral bioavailability. *Heliyon* **2021**, *7*, e07722. [CrossRef]
12. Menon, R.; Gonzalez, T.; Ferruzzi, M.; Jackson, E.; Winderl, D.; Watson, J. Oats—from farm to fork. *Adv. Food Nutr. Res.* **2016**, *77*, 1–55. [PubMed]
13. Scott, M.B.; Styring, A.K.; McCullagh, J.S.O. Polyphenols: Bioavailability, microbiome interactions and cellular effects on health in humans and animals. *Pathogens* **2022**, *11*, 770. [CrossRef]
14. Serra, V.; Salvatori, G.; Pastorelli, G. Dietary polyphenol supplementation in food producing animals: Effects on the quality of derived products. *Animals* **2021**, *11*, 401. [CrossRef]
15. Stover, M.G.; Watson, R.R. Polyphenols in foods and dietary supplements: Role in veterinary medicine and animal health. In *Polyphenols in Human Health and Disease*; Academic Press: Cambridge, MA, USA, 2014; Chapter 1; pp. 3–7.
16. Abdel-Aal, E.S.; Young, J.C.; Rabalski, I. Anthocyanin composition in black, blue, pink, purple and red cereal grains. *J. Agric. Food Che.* **2006**, *54*, 4696–4704. [CrossRef] [PubMed]
17. Varga, M.; Jójárt, R.; Fónad, P.; Mihály, R.; Palágyi, A. Phenolic composition and antioxidant activity of colored oats. *Food Chem.* **2018**, *268*, 153–161. [CrossRef] [PubMed]
18. Fontaneli, R.S.; dos Santos, H.P.; Fontaneli, R.S.; Oliveira, J.T.; Lehmen, R.I.; Dreon, G. Winter forage grasses. In *Forrageiras Para Integração Lavoura-Pecuária-Floresta NA Região Sul-Brasileira*; Fontaneli, R.S., dos Santos, H.P., Fontaneli, R.S., Eds.; EMBRAPA: Brasilia, Brazi, 2012.

19. Derenevicz Faisca, L.; Peres, M.T.P.; Fernandes, S.R.; Bonnet, O.J.F.; Batista, R.; Deiss, L.; Monteiro, A.G. A new insight about the selection and intake of forage by ewes and lambs in different production systems on pasture. *Small Rumin. Res.* **2023**, *221*, 106949. [CrossRef]
20. Vega-García, J.I.; López-González, F.; Estrada-Flores, J.G.; Flores-Calvete, G.; Prospero-Bernal, F.; Arriaga-Jordán, C.M. Black oat (*Avena strigosa* Schreb.) grazing or silage for small-scale dairy systems in the highlands of central Mexico. Part I. Crop and dairy cow performance. *Chil. J. Agric. Res.* **2020**, *80*, 515–525. [CrossRef]
21. Vonz, D.; Menezes, L.F.G.; Paris, W.; Kuss, F.; Silveira, M.F.; Venturini, T.; Stanqueviski, F.; Boito, B. Performance of steers fed on pasture receiving different seeding rates of vetch in an integrated crop-livestock system. *Span. J. Agric. Res.* **2021**, *19*, e06SC01. [CrossRef]
22. Silva, T.B.P.; Del Valle, T.A.; Ghizzi, L.G.; Silva, G.G.; Gheller, L.S.; Marques, J.A.; Dias, M.S.S.; Nunes, A.T.; Grigoletto, N.T.S.; Takiya, C.S.; et al. Partial replacement of corn silage with whole-plant soybean and black oat silages for dairy cows. *J. Dairy Sci.* **2021**, *104*, 9842–9852. [CrossRef]
23. Bernardes, G.M.C.; Carvalho, S.; Pires, C.C.; Motta, J.H.; Teixeira, W.S.; Borges, L.I.; Fleig, M.; Pilecco, V.M.; Farinha, E.T.; Venturini, R.S. Consumption, performance and economic analysis of the feeding of lambs finished in feedlot as the use of high-grain diets. *Arq. Bras. Med. Vet. Zootec.* **2015**, *67*, 1684–1692. [CrossRef]
24. Bernardes, G.M.C.; Carvalho, S.; Venturini, R.S.; Teixeira, W.S.; Motta, J.H.; Borges, L.I.; Rosa, J.S.; Pesamosca, A.C.; Cocco, A.; Mello, V.L.; et al. Carcass characteristics and tissue composition of the meat of feedlot lambs fed high-grain diets. *Semin. Ciências Agrárias* **2018**, *39*, 2637–2646.
25. Karakuş, F. Weaning stress in lambs. *J. Int. Sci. Pub. Agric. Food* **2014**, *2*, 165–170.
26. Lynch, E.; McGee, M.; Earley, B. Weaning management of beef calves with implications 322 for animal health and welfare. *J. Appl. Anim. Res.* **2019**, *47*, 167–175. [CrossRef]
27. Freitas-de-Melo, A.; Orihuela, A.; Hötzel, M.J.; Ungerfeld, R. What do we know and need to know about weaning in sheep? An overview of weaning practises, stress and welfare. *Front. Anim. Sci.* **2022**, *3*, 823188.
28. Freitas-de-Melo, A.; Ungerfeld, R. Destete artificial en ovinos: Respuesta de estrés y bienestar animal. *Rev. Mex. Cienc. Pecu.* **2016**, *7*, 361–375. [CrossRef]
29. Damián, J.P.; Hötzel, M.J.; Banchero, G.; Ungerfeld, R. Behavioural response of grazing lambs to changes associated with feeding and separation from their mothers at weaning. *Res. Vet. Sci.* **2013**, *95*, 913–918. [CrossRef]
30. Freitas-de-Melo, A.; Ungerfeld, R.; Pérez-Clariget, R. Behavioral pattern in Texel × Corriedale terminal crossbreeding: Maternal behavior score at birth, lambs' feeding behaviors, and behavioral responses of lambs to abrupt weaning. *J. Vet. Behav.* **2019**, *30*, 9–15. [CrossRef]
31. Schichowski, C.; Moors, E.; Gauly, M. Effects of weaning lambs in two stages or by abrupt separation on their behavior and growth rate. *J. Anim. Sci.* **2008**, *86*, 220–225. [CrossRef]
32. Selaive-Villarroel, A.B.; Maciel, M.B.; de Oliveira, N.M. Effects of weaning age and weight on lamb growth rate of Morada Nova breed raised in a tropical extensive production system. *Ciência Rural. Santa Maria* **2008**, *38*, 784–788. [CrossRef]
33. Xu, Y.; Yin, F.; Wang, J.; Wu, P.; Qiu, X.; He, X.; Xiao, Y.; Gan, S. Effect of tea polyphenols on intestinal barrier and immune function in weaned lambs. *Front. Vet. Sci.* **2024**, *11*, 1361507. [CrossRef]
34. Russel, A. Body condition scoring of sheep. In *Sheep and Goat Practice*; Boden, E., Ed.; Bailliere Tindall: Philadelphia, PA, USA, 1991; p. 3.
35. National Research Council (NRC). *Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids*; The National Academy Press: Washington, DC, USA, 2007; p. 293.
36. Association of Official Analytical Chemists (AOAC). *Official Methods of Analysis*, 18th ed.; AOAC: Arlington, VA, USA, 2006.
37. Antunovic, Z.; Novoselec, J.; Klir Šalavardic, Ž.; Steiner, Z.; Šperanda, M.; Jakobek Barron, L.; Ronta, M.; Pavic, V. Influence of red corn rich in anthocyanins on productive traits, blood metabolic profile, and antioxidative status of fattening lambs. *Animals* **2022**, *12*, 612. [CrossRef]
38. INRAE-CIRAD-AFZ. Feed tables composition and nutritive values of feeds for cattle, sheep, goats, pigs, poultry, rabbits, horses and salmonids. Available online: https://www.feedtables.com (accessed on 10 July 2024).
39. Jakobek, L.; Matić, P.; Ištuk, J.; Barron, A.R. Study of interactions between individual phenolics of aronia with barley β-glucan. *Pol. J. Food Nutr. Sci.* **2021**, *71*, 187–196. [CrossRef]
40. Antunović, Z.; Domaćinović, M.; Šperanda, M.; Liker, B.; Mioč, B.; Šerić, V.; Šperanda, T. Effect of roasted cereals and soybean in feed mixtures on fattening and slaughter traits as well as blood composition in fattening lambs. *Arch. Tierz.* **2009**, *52*, 512–526. [CrossRef]
41. CIE (Commission Internationale de l'Eclairage). *Colorimetry, Official Recommendations of the International Commission on Illumination*; Publication CIE No. 15 (E-1.3.1); Bureau Central dela CIE: Paris, France, 1976.
42. Sierra, I. Contributions to the study of the Belgian White_Landrace cross: Productive characters, carcass quality and meat quality. *Rev. Inst. Econom. Prod. Ebro.* **1973**, *16*, 43–48.
43. Qwele, K.; Hugo, A.; Oyedemi, S.O.; Moyo, B.; Masika, P.J.; Muchenje, V. Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. *Meat Sci.* **2013**, *93*, 455–462. [CrossRef] [PubMed]

44. Liu, F.; Dai, R.; Zhu, J.; Li, X. Optimizing color and lipid stability of beef patties with a mixture design incorporating with tea catechins, carnosine, and α-tocopherol. *J. Food Eng.* **2010**, *98*, 170–177. [CrossRef]
45. TIBCO. *Statistica, version 13.3.0*; TIBCO Software Inc.: Palo Alto, CA, USA, 2017.
46. Lepherd, M.L.; Canfield, P.J.; Hunt, G.B.; Bosward, K.L. Haematological, biochemical and selected acute phase protein reference intervals for weaned female Merino lambs. *Aust. Vet. J.* **2009**, *87*, 5–11. [CrossRef]
47. Latimer, K.S.; Maheffey, E.A.; Prasse, K.W. Duncan and Prasse's Veterinary Labaratory Medicine: Clinical Pathology, 4th ed. In *Plumb's Veterinary Drug Handbook*, 5th ed.; Wiley-Blackwell: Hoboken, NJ, USA, 2003; pp. 1241–1249.
48. Kaneko, J.J.; Harvey, J.W.; Bruss, M.L. *Clinical Biochemistry of Domestic Animals*, 6th ed.; Elsevier Academic Press: Amsterdam, The Netherlands, 2008; p. 963.
49. Antunović, Z.; Novoselec, J.; Šperanda, M.; Domaćinović, M.; Đidara, M. Monitoring nutritional status of lambs in organic breeding. *Krmiva* **2010**, *52*, 27–34.
50. Antunović, Z.; Klir Šalavardić, Ž.; Steiner, Z.; Đidara, M.; Drenjančević, M.; Ronta, M.; Pavić, V.; Jakobek Barron, L.; Novoselec, J. Meat quality, metabolic profile and antioxidant status of lambs fed on seedless grape pomace (*Vitis vinifera* L.). *Ann. Anim. Sci.* **2023**, *23*, 809–818. [CrossRef]
51. Tian, S.; Sun, Y.; Chen, Z.; Yang, Y.; Wang, Y. Functional properties of polyphenols in grains and effects of physicochemical processing on polyphenols. *Hindawi J. Food Qual.* **2019**, *2019*, 2793973. [CrossRef]
52. Jiao, X.; Wang, Y.; Lin, Y.; Lang, Y.; Li, E.; Zhang, X.; Zhang, Q.; Feng, Y.; Meng, X.; Li, B. Blueberry polyphenols extract as a potential prebiotic with anti-obesity effects on C57BL/6 J mice by modulating the gut microbiota. *J. Nutr. Biochem.* **2019**, *64*, 88–100. [CrossRef] [PubMed]
53. Tian, X.; Li, J.; Luo, Q.; Wang, X.; Wang, T.; Zhou, D.; Xie, L.; Ban, C.; Lu, Q. Effects of purple corn Anthocyanin on growth performance, meat quality, muscle antioxidant status, and fatty acid profiles in goats. *Foods* **2022**, *11*, 1255. [CrossRef] [PubMed]
54. Orzuna-Orzuna, J.F.; Dorantes-Iturbide, G.; Lara-Bueno, A.; Mendoza-Martínez, G.D.; Miranda-Romero, L.A.; Hernández-García, P.A. Growth performance, carcass characteristics, and blood metabolites of lambs supplemented with a polyherbal mixture. *Animals* **2021**, *11*, 955. [CrossRef] [PubMed]
55. Cimmino, R.; Barone, C.M.A.; Claps, S.; Varrivvhio, E.; Rufrano, D.; Caroprese, M.; Albenzi, M.; De Palo, P.; Neglia, G. Effect of dietary supplementation with polyphenols on meat quality in Saanen goat kids. *BMC Vet. Res.* **2018**, *14*, 181. [CrossRef] [PubMed]
56. Muela, E.; Alonso, V.; Campo, M.M.; Sañudo, C.; Beltrán, J.A. Antioxidant diet supplementation and lamb quality throughout preservation time. *Meat Sci.* **2014**, *98*, 289–295. [CrossRef]
57. Kholif, A.E.; Hassan, A.A.; El Ashry, G.M.; Bakr, M.H.; El-Zaiat, H.M.; Olafadehan, O.A.; Matloup, O.H. Phytogenic feed additives mixture enhances the lactational performance, feed utilization and ruminal fermentation of Friesian cows. *Anim. Biotechnol.* **2020**, *32*, 708–718. [CrossRef]
58. Fraga, C.G.; Oteiza, P.I. Dietary flavonoids: Dietary flavonoids: Role of (−)-epicatechin and related procyanidins in cell signaling. *Free Rad. Biol. Med.* **2011**, *51*, 813–823. [CrossRef]
59. Antunović, Z.; Novoselec, J.; Klir, Ž. Hematological parameters in ewes during lactation in organic farming. *Poljoprivreda* **2017**, *23*, 46–52. [CrossRef]
60. Teng, X.; Zhang, X.; Liu, Y.; Wang, H.; Luo, J.; Luo, Y.; An, P. Effects of dietary polyphenol supplementation on iron status and erythropoiesis: A systematic review and meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **2021**, *114*, 780–793.
61. Youdim, K.A.; Shukitt-Hale, B.; MacKinnon, S.; Kalt, W.; Joseph, J.A. Polyphenolics enhance red blood cell resistance to oxidative stress: In vitro and in vivo. *Biochim. Biophys. Acta* **2000**, *1523*, 117–122. [CrossRef]
62. Antunović, Z.; Mioč, B.; Klir Šalavardić, Ž.; Širić, I.; Držaić, V.; Đidara, M.; Novoselec, J. The effect of lactation stage on the hematological and serum-related biochemical parameters of the Travnik pramenka ewe. *Poljoprivreda* **2021**, *27*, 56–62. [CrossRef]
63. Balcells, J.; Aris, A.; Serrano, A.; Seradj, A.R.; Crespo, J.; Devant, M. Effects of an extract of plant flavonoids (Bioflavex) on rumen fermentation and performance in heifers fed high-concentrate diets. *J. Anim. Sci.* **2012**, *90*, 4975–4984. [CrossRef]
64. Mbuh, J.V.; Mbwaye, J. Serological changes in goats experimentally infected with Fasciola gigantica in Buea sub-division of S.W.P. Cameroon. *Vet. Parasitol.* **2005**, *131*, 255–259. [CrossRef] [PubMed]
65. Giannenas, I.; Skoufos, J.; Giannakopoulos, C.; Wiemann, M.; Gortzi, O.; Lalas, S.; Kyriazakis, I. Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. *J. Dairy Sci.* **2011**, *94*, 5569–5577. [CrossRef]
66. Butt, M.S.; Tahir-Nadeem, M.; Khan, M.K.I.; Shabir, R.; Butt, M.S. Oat: Unique among the cereals. *Eur. J. Nutr.* **2008**, *47*, 68–79. [CrossRef]
67. Silanikove, N.; Tiomokin, D. Toxicity induced by poultry litter consumption: Effect on parameters reflecting liver function in beef cows. *Anim. Prod.* **1992**, *54*, 203–209. [CrossRef]
68. Peng, K.; Shirley, D.C.; Xu, Z.; Huang, Q.; McAllister, T.A.; Chaves, A.V.; Acharya, S.; Liu, C.; Wang, S.; Wang, Y. Effect of purple prairie clover (*Dalea purpurea* Vent.) hay and its condensed tannins on growth performance, wool growth, nutrient digestibility, blood metabolites and ruminal fermentation in lambs fed total mixed rations. *Anim. Feed Sci. Technol.* **2016**, *222*, 100–110. [CrossRef]
69. Serra, A.; Macià, A.; Romero, M.-P.; Anglès, N.; Morelló, J.R.; Motilva, M.J. Distribution of procyanidins and their metabolites in rat plasma and tissues after an acute intake of hazelnut extract. *Food Funct.* **2011**, *2*, 562–568. [CrossRef] [PubMed]
70. Zhang, R.F.; Zeng, Q.S.; Deng, Y.Y.; Zhang, M.W.; Wei, Z.C.; Tang, X.J. Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. *Food Chem.* **2012**, *136*, 11691176. [CrossRef]

71. Ng, K.R.; Lyu, X.; Mark, R.; Chen, W.N. Antimicrobial and antioxidant activities of phenolic metabolites from flavonoid-producing yeast: Potential as natural food preservatives. *Food Chem.* **2019**, *70*, 123–129. [CrossRef]
72. Callcott, E.T.; Santhakumar, A.B.; Luo, J.; Blanchard, C.L. Terapeutic potential of rice-derived polyphenols on obesity-related oxidative stress and inflammation. *J. Appl. Biomed.* **2018**, *16*, 255–262. [CrossRef]
73. Liu, S.; You, L.; Zhao, Y.; Chang, X. Wild lonicera caerulea berry polyphenol extract reduces cholesterol accumulation and enhances antioxidant capacity in vitro and in vivo. *Food Res. Int.* **2018**, *107*, 73–83. [CrossRef] [PubMed]
74. Cuevas, A.; Saavedra, N.; Salazar, L.; Abdalla, D. Modulation of immune function by polyphenols: Possible contribution of epigenetic factors: Possible con-tribution of epigenetic factors. *Nutrients* **2013**, *5*, 2314–2332. [CrossRef]
75. Bañón, S.; Méndez, L.; Almela, E. Effects of dietary rosemary extract on lamb spoilage under retail display conditions. *Meat Sci.* **2012**, *90*, 579–583. [CrossRef]
76. Sañudo, C.; Santolaria, M.P.; Maria, G.; Osorio, M.; Sierra, I. Influence of carcass weight on instrumental and sensory lamb meat quality in intensive production systems. *Meat Sci.* **1996**, *42*, 195–202. [CrossRef] [PubMed]
77. Dalle, Z.O. Perception of rabbit meat quality and major factors influencing the rabbit carcass and meat quality. *Livest. Prod. Sci.* **2002**, *75*, 11–32. [CrossRef]