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Effects of Selenium-Biofortified Maize and Soybean in Maternal and Lamb Diets on Tissue Selenium Deposition, Growth Performance, and Meat Quality in Lambs

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Abstract

This study examined tissue selenium (Se) deposition and muscle Se speciation in lambs receiving Se-biofortified maize and soybean through maternal and direct feeding. Thirty Merinolandschaf ewes (10 per group) received a control mixture containing inorganic Se (Group I; 0.298 mg/kg dry matter (DM)) or biofortified mixtures (Groups II and III; 0.312 and 0.476 mg/kg DM). Ewes received these diets during late gestation and lactation; their lambs suckled and had free access to the same mixtures from birth to slaughter (n = 8, 9, and 12, respectively). Individual tissue Se was determined by inductively coupled plasma mass spectrometry (ICP–MS), and speciation in pooled muscle by high-performance liquid chromatography–ICP–MS (HPLC–ICP–MS). Muscle Se was higher in Groups II and III than in Group I (0.692 and 0.742 vs. 0.329 mg/kg DM; $p < 0.001$). Heart and liver Se were also higher ($p < 0.001$). Growth, dressing percentage, and colour were unaffected; water-holding capacity tended to differ ($p = 0.059$). Selenomethionine predominated in pooled muscle, accounting for 32.4%, 51.2%, and 52.7% of total Se, respectively. Combined feeding was associated with higher tissue Se without significant changes in growth or measured meat quality. Maternal and direct dietary effects could not be separated.

Keywords: selenium biofortification; lamb meat; selenium speciation; selenomethionine; HPLC–ICP–MS; meat quality traits

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1. Introduction

Demand for foods of high nutritional value has increased, yet essential micronutrient deficiencies remain widespread in humans and animals [1,2]. Selenium (Se) is incorporated into selenoproteins involved in antioxidant defence and cellular redox regulation [3,4]. In skeletal muscle, Se-dependent glutathione peroxidases limit post-mortem oxidative damage by reducing hydrogen peroxide and lipid hydroperoxides [5,6]. Dietary Se may therefore affect Se deposition in muscle and the nutritional value and oxidative stability of meat. Feed Se content largely reflects soil Se availability. Many European soils, including those in continental Croatia, have low Se availability, limiting its accumulation in locally produced crops [7,8]. The causes and consequences of Se deficiency in small ruminants

were reviewed by Novoselec et al. [9]. Dietary supplementation is therefore commonly used to meet Se requirements and increase deposition in edible tissues.

Se metabolism and tissue retention depend strongly on its chemical form. Inorganic sodium selenite and selenate enter the hydrogen selenide pool for selenoprotein synthesis, whereas excess Se is methylated and excreted. Organic Se, particularly selenomethionine (SeMet), follows methionine pathways and may replace methionine in body proteins, increasing skeletal-muscle retention [5,10]. Consequently, organic sources generally produce greater muscle Se deposition than inorganic forms [5,11,12]. In lambs, Se-enriched yeast and SeMet similarly produce greater muscle deposition than sodium selenite, particularly at higher Se concentrations [5,12,13].

Plants convert part of the applied inorganic Se into organically bound compounds, including SeMet and other selenoamino acids. Foliar application is generally more efficient than soil application because it bypasses soil adsorption and immobilization [14]. Simultaneous biofortification of maize and soybean, major energy and protein sources in sheep diets, may provide plant-derived organic Se through several feed ingredients. Maize and soybean were selected for Se biofortification because they represent major components of concentrate mixtures, providing primarily energy and protein, respectively. Biofortifying both ingredients enabled Se to be incorporated through the principal feed components rather than supplied solely as an inorganic mineral supplement. This approach was intended to create a practical feeding strategy in which Se was delivered within the feed matrix of commonly used dietary ingredients. This approach allowed us to evaluate maize and soybean together as biofortified feed sources, but not to determine whether their combination was more effective than either ingredient alone. Maternal Se is transferred through the placenta and subsequently through colostrum and milk; supplementation during late gestation and lactation may therefore improve offspring Se status and tissue deposition [15,16]. Feeding Se-biofortified maize and soybean may combine maternal transfer with the greater retention of organically bound Se. Greater muscle Se deposition may enhance antioxidant protection and meat quality [5,6]. Oxidation of lipids and myoglobin contributes to off-flavours, discoloration, and reduced shelf life, whereas protein oxidation may impair water-holding capacity [17–19]. Organic Se has been associated with increased muscle Se, antioxidant capacity, and colour stability [5], but effects on carcass traits, pH, colour, and water-holding capacity remain inconsistent [5,11,20].

Total tissue Se concentration alone does not provide information on the chemical forms in which Se is retained. SeMet may enter structural muscle proteins, whereas selenocysteine is mainly associated with functional selenoproteins. High-performance liquid chromatography coupled with inductively coupled plasma mass spectrometry (HPLC–ICP–MS) separates and quantifies individual species, providing a more complete assessment of muscle Se deposition than total Se alone [21,22].

Although the main metabolic pathways of inorganic and organic Se are well described [4], less is known about tissue Se deposition when Se is supplied through crops biofortified during growth. Unlike direct supplementation with inorganic salts or Se-enriched yeast, this approach introduces Se through maize and soybean following foliar sodium selenate application. The present study evaluates this crop-based feeding strategy by combining individual tissue Se measurements with exploratory Se speciation in pooled lamb muscle. This study evaluated the combined effects of feeding Se-biofortified maize and soybean to ewes during late gestation and lactation and providing their lambs access to the same diets from birth to slaughter on tissue Se deposition, growth performance, carcass characteristics, and selected meat-quality traits. Muscle Se speciation was explored using HPLC–ICP–MS.

2. Materials and Methods

2.1. Experimental Animals, Design, and Housing

The research was conducted at the Sičaja family farm in Gašinci, Osijek-Baranja County, Croatia, during 2023–2024. It began with ewes in late gestation in 2023 and continued through lambing and lamb rearing until slaughter and tissue sampling in 2024.

All animal procedures were reviewed and approved by the Bioethical Commission for Animal Research, Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek (Class: 644-01/22-01/03; File No.: 2158-94-02-22-16; 30 September 2022). Procedures were conducted in accordance with the Croatian Animal Protection Act (Official Gazette Nos. 102/17 and 32/19) and relevant national regulations governing animal welfare.

Thirty clinically healthy Merinolandschaf ewes in good body condition (BCS 3.5–4.0) were selected during the final third of gestation before their fourth lambing. Pregnancy was confirmed using a Tringa 50S ultrasound scanner (PIE Medical, Maastricht, The Netherlands). The sample size was determined based on previous similar studies and the number of animals available. A 10-day adaptation period preceded the experiment. The five-month experiment comprised approximately two months of late gestation and three months of lactation and lamb rearing. Ewes were allocated to three dietary groups (n = 10 each), balancing the groups as closely as possible for body condition and body weight. Among the 32 born lambs, two in Group I and one in Group II were stillborn, leaving 29 lambs in total (n = 8, 9, and 12, for Group I, II and III); the larger Group III resulted from two twin births. Sex distribution was balanced overall (15 males and 14 females) and comparable among groups (4:4, 5:4, and 6:6, respectively). Lambs had free access from birth to slaughter to the same treatment-specific feed mixture offered to their dams, while hay and water were available ad libitum. Individual feed intake of lambs was not recorded because lambs were group-housed with their dams. Ewes and their lambs were maintained on the same dietary treatment to evaluate a continuous Se feeding strategy from late gestation to lamb slaughter, without separating maternal and direct dietary effects. Access to the feed mixture from birth does not imply measurable consumption from the first day of life; the onset and amount of individual lamb feed intake were not determined. Each group was housed in a separate pen with approximately 1 m^2 of lying area per animal and continuous access to water. All three pens were located in the same barn, with the same bedding, cleaning routine, ventilation, and lighting conditions. Lambs were slaughtered at market weight at 106.86 $\pm$ 12.44 days of age (mean $\pm$ SD; range, 73–138 days). Semimembranosus muscle and internal organs were collected from all lambs for meat-quality and Se analyses.

2.2. Experimental Diets and Feeding System

Diets offered to ewes during late gestation and lactation and subsequently to their lambs consisted of hay and a feed mixture. Maize and soybean were biofortified by foliar application of sodium selenate at rates equivalent to 0, 10, 20, or 30 g of Se per hectare, at maize tasseling and soybean flowering, respectively. The application rates were adapted from previous studies on Se biofortification of cereals [14] and soybean [23], taking into account the experience of the project collaborators. After harvest and thermal processing of soybean, Se concentrations were determined by laboratory analysis in all feed batches. Conventional and biofortified batches were then selected and combined with the remaining ingredients to formulate feed mixtures with the intended Se concentrations.

Ewes received 700 g of the respective feed mixture per animal daily. In Group I, the mineral–vitamin premix contained 44 mg Se/kg as sodium selenite and was included at 0.5%, supplying 0.220 mg Se/kg feed mixture on an as-fed basis. Conventional feed ingredients provided additional background Se. In Group II, biofortified maize and extruded soybean contained 0.270 and 1.150 mg Se/kg DM, respectively, and were included at 51% and 14% of the feed mixture on an as-fed basis. All ingredient inclusion rates remained unchanged, but the premix contained no added Se. In Group III, biofortified maize and extruded soybean contained 0.350 and 1.490 mg Se/kg DM, respectively. The inclusion rates of all ingredients were the same across the three groups. As in Group II, the premix used in Group III contained no added Se. Se concentrations in biofortified maize grain and soybean were measured before feed mixture formulation. Batches with different Se concentrations were combined to achieve the intended Se levels in Groups II and III. The total inclusion rates of maize and soybean were identical across all three groups (Table 1). The expected Se concentrations were used for formulation, whereas the analysed concentrations of the prepared feed mixtures are reported in Table 2. Lambs had free access to the corresponding feed mixtures from birth to slaughter; hay and water were available *ad libitum*. Se exposure was described using the feeding treatments and measured Se concentrations in the feed mixtures and hay. However, individual Se intake could not be determined because individual feed consumption was not recorded. During the experimental period, a new batch of each feed mixture was prepared approximately once per month. One 10-g sample was collected from each of five independently prepared batches of each feed mixture, resulting in five samples per treatment. In addition, one representative 10-g hay sample was collected. Samples were dried at 60 °C and ground through a 1-mm screen (Microtron MB 550; Kinematica, Lucerne, Switzerland). Proximate composition was analysed using standard methods [24]. Crude protein was calculated from Kjeldahl nitrogen using a factor of 6.25 [25], and crude fat was determined according to Onwuka [26] using an Extraction System B-811 (BÜCHI Labortechnik AG, Flawil, Switzerland). Crude fibre was determined by the Weende method and ash by incineration at 550 °C for 6 h. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the FibreBag system (C. Gerhardt GmbH & Co. KG, Königswinter, Germany). NDF followed Van Soest et al. [27], whereas ADF and ADL followed AOAC Official Method 973.18 [24]. Ingredient and chemical composition are presented in Table 1 and analysed Se concentrations in Table 2.

Analysed Se concentration differed among feed mixtures ($p = 0.004$): the Se concentration was significantly higher in Group III than in Groups I and II, whereas no significant difference was observed between Groups I and II. The hay contained 0.018 mg Se/kg DM.

Table 1. Ingredients and chemical composition of the experimental feed mixtures and hay.

Component, %	Group			Hay
	I	II	III	
Maize	51.0 *	51.0 **	51.0 **	
Barley	17.0	17.0	17.0	
Cracked wheat	10.0	10.0	10.0	
Soybean meal	5.5	5.5	5.5	
Extruded soybean	14.0 *	14.0 **	14.0 **	
Limestone	1.1	1.1	1.1	
Monocalcium phosphate	0.6	0.6	0.6	
Animal salt	0.3	0.3	0.3	
Mineral premix	0.5 [1]	0.5 [2]	0.5 [2]	

Table 1. *Cont.*

Component, %	Group I	Group II	Group III	Hay
	Chemical composition, %			
Dry matter	90.42	90.83	90.90	93.29
Crude protein	13.28	13.01	12.98	9.26
Crude fat	5.51	5.63	5.68	1.28
Crude fibre	2.82	2.74	2.62	24.03
Ash	4.60	4.75	4.89	6.30
NDF	35.64	36.80	37.21	49.07
ADF	4.75	4.69	4.41	32.81
ADL	2.52	1.67	1.67	5.61
Metabolizable energy, MJ/kg DM	12.37	12.37	12.37	7.48

* Conventional maize grain and soybean products. ** Se-biofortified maize grain and soybean products. [1] Mineral–vitamin premix with supplemental Se as sodium selenite. [2] Mineral–vitamin premix without added Se.

Table 2. Expected and analysed selenium concentrations of the experimental feed mixtures (mg/kg DM).

Variable	Se Concentration (mg/kg DM) Group			SEM	*p*-Value
	I	II	III		
Expected	0.290	0.330	0.420	-	-
Analysed total Se concentration	0.298 [b]	0.312 [b]	0.476 [a]	0.033	0.004

Analysed values are means of five independently prepared batches per group. [a,b] Means with different superscripts differ ($p < 0.05$). SEM, standard error of the mean; DM, dry matter. Group I, sodium-selenite control; Group II, Se-biofortified feed with comparable Se; Group III, Se-biofortified feed with higher Se.

2.3. Slaughter Procedures and Carcass Evaluation

Body weight was recorded using an EOS 150K50XL animal scale (Kern & Sohn GmbH, Balingen, Germany). Lambs were slaughtered commercially by exsanguination after severing the major neck vessels (*vena jugularis externa* and *arteria carotis communis*), and carcasses were skinned. The gastrointestinal tract, visceral organs (lungs, trachea, heart, spleen, and liver), skin, lower legs, and carcass were removed and weighed separately. Three carcass lengths were measured from the pubic bone (*os pubis*) to the atlas, first rib, and last rib, respectively. Chest and hindquarter circumferences, hind leg length (*tuber calcanei* to *tuber ischiadicum*), and maximum hind leg circumference were also recorded.

Dressing percentage was calculated as:

$$\text{Dressing percentage (\%)} = [\text{hot carcass weight (kg)}/\text{live weight at slaughter (kg)}] \times 100$$

2.4. Total Selenium Determination in Lamb Semimembranosus Muscle

Total Se was determined in 0.2-g portions of lyophilised semimembranosus muscle. Samples were pre-digested overnight in DigiPREP tubes with 2.5 mL concentrated nitric acid (HNO_3), followed by 1 mL hydrogen peroxide (H_2O_2). DigiPREP digestion involved heating to 65 °C over 30 min and holding for 210 min. Digests were adjusted to 4% HNO_3 (*v*/*v*) and analysed by inductively coupled plasma–mass spectrometry (ICP–MS; Agilent 7700, Agilent Technologies, Tokyo, Japan). Se was quantified by standard addition at four levels. Samples were analysed in duplicate with procedural blanks and certified reference material SELM-1 (National Research Council Canada, Ottawa, ON, Canada). Results are expressed as mg Se/kg dry matter.

2.5. Selenium Speciation in Lamb Semimembranosus Muscle

Se speciation was performed at IPREM, Université de Pau et des Pays de l'Adour, Pau, France, using one pooled muscle sample per group. Three independent 0.2-g subsamples from each pool underwent the complete preparation procedure, and each digest was analysed in duplicate by HPLC–ICP–MS. Results are expressed as mean ± SD of the three prepared subsamples; relative species contributions were calculated against total Se in the corresponding pool. The quantified Se species did not account for 100% of total Se. The unquantified fraction may reflect incomplete extraction and/or additional Se species not determined by the applied method. One Group I sample, identified as an outlier by modified Z-score > 3, was excluded from pooling but retained in the analysis of individual total Se concentrations.

Selenocysteine (SeCys) and selenomethionine (SeMet) were determined in 0.2-g pooled-muscle subsamples. Samples were extracted three times with fresh 5-mL water portions by sonication for 1 h and centrifuged at 4000× *g* (5804R; Eppendorf, Hamburg, Germany). The remaining pellet was suspended in 2 mL 0.1 M TRIS buffer (pH 7.5). Disulfide bridges were reduced with 30 µL dithiothreitol (DTT; 0.2 M in TRIS buffer), and 50 µL iodoacetamide (IAM; 0.5 M in TRIS buffer) was added. After alkylation, 150 µL DTT was added and the mixture shaken for 1 h to remove excess IAM. Subsequently, 10 mL TRIS buffer and 1 mL protease XIV solution (30 mg enzyme in 2 mL 100 mM TRIS, pH 7.5) were added. After 2 h at 37 °C, another 1 mL protease solution was added and incubation continued overnight.

After digestion, samples were centrifuged, filtered through a 2-kDa cut-off filter, and analysed by HPLC–ICP–MS using an Agilent 1200 HPLC coupled to an Agilent 7700 ICP–MS (Agilent, Tokyo, Japan) with platinum cones and a 1-mm injector. Separation used an Acclaim C8 column (4.6 × 250 mm, 5 µm, 120 Å; Fisher Scientific, Illkirch, France). Mobile phases were 0.2% formic acid in water (A) and 0.2% formic acid in methanol (B). The programme was 5% B at 0–5 min; 5–50% B at 5–15 min; 50% B at 15–20 min; 50–5% B at 20–22 min; and 5% B at 22–25 min, at 0.9 mL/min. SeCys and SeMet were quantitatively determined using the standard addition method with SeCys-CAM and SeMet standards at three concentration levels. Blank samples and the certified reference material VORM-1 (National Research Council of Canada) were included for analytical quality control.

2.6. Total Selenium Determination in Lamb Internal Organs, Experimental Feed Mixtures, and Hay

Total Se was determined in 0.2-g portions of fresh heart, liver, and kidney and dried, ground samples from each feed-mixture batch and hay. Samples were pre-digested overnight in 100-mL vessels with 6 mL concentrated HNO_3 (trace-metal grade; Fisher Scientific). After adding 2 mL H_2O_2 (trace-metal grade; Fisher Scientific), microwave digestion was performed using a SINEO system (SINEO Microwave Chemistry Technology [Shanghai] Co., Ltd., Shanghai, China). Digests were diluted to 25 mL and analysed by ICP–MS (Agilent 7800; Agilent Technologies, Tokyo, Japan). Organ concentrations are expressed as mg Se/kg fresh weight (FW), and feed-mixture and hay concentrations as mg Se/kg dry matter (DM). Accuracy was assessed with ERM®-BB184 bovine muscle: 417.3 µg/kg was measured against the certified 450 ± 40 µg/kg (92.7% recovery).

2.7. Meat Quality Evaluation

Muscle pH was measured 45 min postmortem (pH_{45}) in the semimembranosus muscle on the carcass using a Mettler Toledo meter with a spear-tip electrode. A muscle sample was then collected from each lamb and stored at 4 °C; pH was remeasured after 24 h (pH_{24}). Colour was measured at 45 min postmortem on the exposed muscle surface using a

Minolta Chroma Meter CR-410 (Minolta Camera Co., Ltd., Osaka, Japan) under illuminant D65 with a 2° standard observer and a 50-mm aperture, and expressed in the CIE $L^*a^*b^*$ system [28], where L^*, a^*, and b^* represent lightness, redness, and yellowness. Hue angle (h°) and chroma (C*) were calculated as follows:

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

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

Water-holding capacity was determined after 24 h using the filter-paper press method [29]. Approximately 300 mg homogenised muscle was placed between two pre-weighed filter papers and pressed under 3.4 kg for 5 min. After sample removal, the papers were reweighed and water-holding capacity was calculated as follows:

$$\text{WHC (\%)} = \{1 - [(W_2 - W_1)/S]\} \times 100$$

where W_1 is the initial weight of the filter paper, W_2 is its weight after pressing, and S is the initial sample weight.

2.8. Statistical Analysis

Analyses were performed using TIBCO Statistica 14.0.0 [30]. Repeated-measures ANOVA within the general linear model analysed body weight, with dietary treatment as the between-subject factor, time as the within-subject factor, their interaction, and lamb as the repeated subject. Slaughter age, average daily gain, slaughter and carcass traits, carcass measurements, and meat-quality variables were analysed by one-way ANOVA, with dietary treatment as the fixed effect and lamb as the experimental unit. Significant effects were followed by the Tukey–Kramer procedure for unequal group sizes. A sensitivity analysis excluding one lamb from each twin pair did not alter treatment effects or p-values; therefore, all 29 lambs were retained in the final analyses.

Analysed Se concentrations in feed mixtures were evaluated by one-way ANOVA, with dietary treatment as the fixed effect and independently prepared batch as the experimental unit ($n = 5$ per treatment). Expected concentrations were described without statistical analysis. Significant effects were followed by Duncan's multiple range test. Total Se concentrations in muscle, heart, liver, and kidneys were analysed separately by one-way ANOVA, with dietary treatment as the fixed effect and lamb as the experimental unit. Significant effects were followed by Duncan's multiple range test with Kramer's modification for unequal group sizes.

Speciation used one pooled biological muscle sample per group and three separately prepared analytical subsamples per pool; instrumental duplicates were not independent observations. The Group I outlier excluded from pooling remained in the individual total Se analysis. Speciation results are mean ± SD of the three prepared subsamples. Because subsamples from a single pool were not independent biological replicates, groups were not compared inferentially.

Residual normality was assessed by the Shapiro–Wilk test. Animal-level variables, analysed feed-mixture Se, and tissue total Se are presented as means with standard errors (SEM). Differences were significant at $p < 0.05$; $0.05 \leq p < 0.10$ indicated a statistical tendency.

3. Results

Total Se concentrations in the semimembranosus muscle of lambs are presented in Table 3.

Table 3. Total selenium concentration in lamb semimembranosus muscle.

Total Se Concentration (mg/kg DM) Group			SEM	*p*-Value
I	II	III		
0.329 [a]	0.692 [b]	0.742 [b]	0.039	<0.001

Group I, sodium-selenite control (n = 8); Group II, Se-biofortified feed with comparable Se (n = 9); Group III, Se-biofortified feed with higher Se (n = 12). [a,b] Means with different superscripts differ ($p < 0.05$). SEM, standard error of the mean; DM, dry matter.

Total muscle Se concentration was significantly higher in Groups II and III than in Group I ($p < 0.001$), whereas Groups II and III did not differ (Table 3).

Se speciation profiles and the relative contributions of the detected Se species in pooled semimembranosus muscle samples are presented in Figures 1 and 2, respectively. Chromatograms indicated SeCys, inorganic Se, and SeMet in every pooled muscle sample, with SeMet predominating (Figure 1); their relative contributions are shown descriptively in Figure 2.

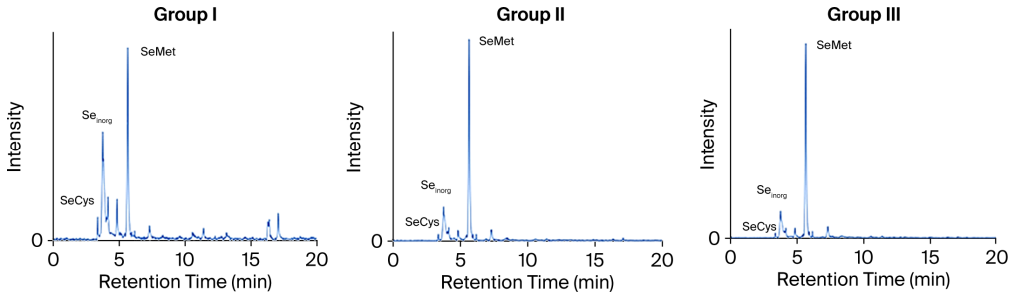

Figure 1. Reversed-phase HPLC–ICP–MS chromatograms with Se-specific detection obtained from the pooled semimembranosus muscle sample prepared for each experimental group. Peaks corresponding to selenocysteine (SeCys), inorganic selenium (Se_{inorg}), and selenomethionine (SeMet) are indicated.

SeMet predominated in all pooled muscle samples, representing 32.4 ± 0.9%, 51.2 ± 0.7%, and 52.7 ± 0.6% of total Se in Groups I, II, and III, respectively (Figure 2). Its contribution was numerically higher in Groups II and III, which had similar values; SeCys and inorganic Se contributed less.

Total Se concentrations in the internal organs are presented in Table 4.

Table 4. Total selenium concentrations in lamb internal organs by dietary group.

Internal Organ	Total Se Concentration (mg/kg FW) Group			SEM	*p*-Value
	I	II	III		
Heart	0.262 [b]	0.376 [a]	0.371 [a]	0.014	<0.001
Liver	0.306 [b]	0.481 [a]	0.523 [a]	0.023	<0.001
Kidney	1.053	1.203	1.225	0.041	0.201

Group I, sodium-selenite control (n = 8); Group II, Se-biofortified feed with comparable Se (n = 9); Group III, Se-biofortified feed with higher Se (n = 12). [a,b] Means with different superscripts differ ($p < 0.05$). SEM, standard error of the mean; FW, fresh weight.

Heart and liver Se concentrations were higher in Groups II and III than in Group I ($p < 0.001$), with no difference between Groups II and III. Kidney Se did not differ ($p = 0.201$) but was numerically highest among the analysed organs (Table 4).

Growth performance and slaughter age, as well as slaughter and carcass traits, are presented in Tables 5 and 6, respectively.

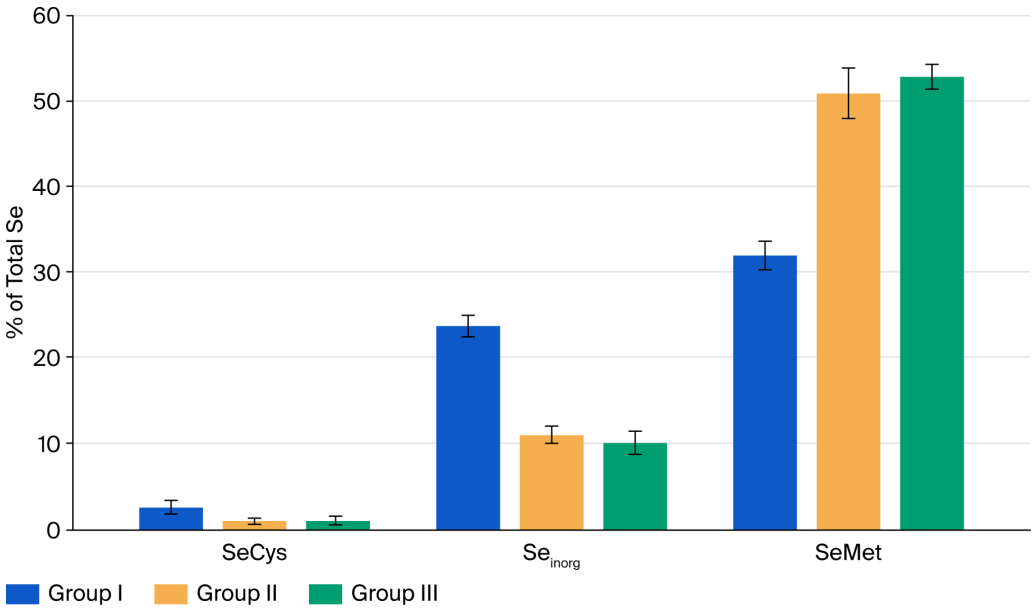

Figure 2. Relative contributions of selenocysteine (SeCys), inorganic selenium (Seinorg), and selenomethionine (SeMet) to total Se in one pooled semimembranosus muscle sample per group. Values are mean ± SD of three separately prepared analytical subsamples from one pooled muscle sample per group. The SD reflects analytical preparation variability, not variability among animals. No statistical comparisons between groups were performed because independent biological replicates were not available.

Table 5. Growth performance of lambs under different maternal and lamb Se feeding treatments.

Indicator	Group			SEM	*p*-Value
	I	II	III		
Initial body weight, kg	4.86	4.21	4.30	0.11	-
Final body weight, kg	32.46	32.95	32.64	0.44	-
Slaughter age, days	106.38	112.00	103.33	2.31	0.294
Overall average daily gain, g/day	250.90	262.32	257.65	4.26	0.607

Group I, sodium-selenite control (n = 8); Group II, Se-biofortified feed with comparable Se (n = 9); Group III, Se-biofortified feed with higher Se (n = 12).

Table 6. Slaughter traits of lambs under different maternal and lamb Se feeding treatments.

Indicators	Group			SEM	*p*-Value
	I	II	III		
Hot carcass weight, kg	16.98	17.00	17.33	0.303	0.869
Dressing percentage, %	52.33	51.64	52.55	0.424	0.668
Visceral organ weight *, kg	1.37	1.40	1.39	0.037	0.952
Skin and lower legs, kg	4.57	4.09	4.11	0.090	0.055
Gastrointestinal tract weight, kg	7.46	7.86	7.61	0.175	0.686

Group I, sodium-selenite control (n = 8); Group II, Se-biofortified feed with comparable Se (n = 9); Group III, Se-biofortified feed with higher Se (n = 12). SEM, standard error of the mean. * Lungs, trachea, heart, spleen, and liver.

For repeated-measures body weight, the effects of dietary treatment, time, and the treatment × time interaction were $p = 0.907$, $p < 0.001$, and $p = 0.489$, respectively. Only initial and final body weights are presented in the table. SEM, standard error of the mean.

Body weight increased over time ($p < 0.001$), without effects of dietary treatment ($p = 0.907$) or treatment × time ($p = 0.489$). Slaughter age and average daily gain did not differ among groups ($p = 0.294$ and $p = 0.607$, respectively; Table 5).

Slaughter traits did not differ among groups. Skin and lower-leg weight tended to differ ($p = 0.055$), with the highest numerical value in Group I (Table 6). Carcass measurements did not differ among groups. Hind leg length tended to differ ($p = 0.069$), with the highest numerical value in Group I and lowest in Group II (Table 7).

Carcass measurements and meat-quality traits are presented in Tables 7 and 8, respectively.

Table 7. Carcass measurements of lambs under different maternal and lamb Se feeding treatments.

Carcass Measurements, cm	Group I	Group II	Group III	SEM	*p*-Value
Carcass length [1]	74.75	75.33	75.17	0.432	0.877
Carcass length [2]	54.44	53.61	54.42	0.561	0.810
Carcass length [3]	28.25	28.28	31.38	1.002	0.322
Chest circumference	64.88	63.28	65.08	0.769	0.598
Hindquarter circumference	51.31	49.44	52.58	0.856	0.314
Hind leg circumference	37.19	36.44	37.04	0.509	0.842
Hind leg length	31.56	29.56	30.38	0.337	0.069

Group I, sodium-selenite control ($n = 8$); Group II, Se-biofortified feed with comparable Se ($n = 9$); Group III, Se-biofortified feed with higher Se ($n = 12$). SEM, standard error of the mean. [1] Pubis to atlas; [2] pubis to first rib; [3] pubis to last rib.

Table 8. Meat quality traits of lambs under different maternal and lamb Se feeding treatments.

Indicators	Group I	Group II	Group III	SEM	*p*-Value
pH_{45}	6.42	6.51	6.58	0.033	0.145
pH_{24}	5.63	5.59	5.65	0.026	0.658
WHC (%)	70.83	68.00	64.83	1.054	0.059
*L**	43.98	44.48	43.83	0.441	0.831
*a**	19.27	18.82	18.91	0.128	0.374
*b**	2.65	2.47	2.36	0.056	0.119
h°	7.84	7.48	7.13	0.165	0.215
C*	19.45	18.98	19.05	0.129	0.330

Group I, sodium-selenite control ($n = 8$); Group II, Se-biofortified feed with comparable Se ($n = 9$); Group III, Se-biofortified feed with higher Se ($n = 12$). SEM, standard error of the mean; WHC, water-holding capacity; *L**, lightness; *a**, redness; *b**, yellowness; h°, hue angle; C*, chroma.

Muscle pH and colour did not differ among groups. Water-holding capacity tended to decrease from Group I to Group III ($p = 0.059$; Table 8).

4. Discussion

4.1. Selenium Deposition in Muscle and Internal Organs

The main finding of this study was the greater Se concentration in lamb muscle when Se was supplied through biofortified maize and soybean rather than as sodium selenite. This was most evident in the comparison between Groups I and II. The analysed Se concentrations of the experimental feed mixtures differed among groups (Table 2), but the concentrations in Groups I and II did not differ significantly (0.298 and 0.312 mg/kg DM, respectively). In contrast, muscle Se concentration differed among groups (Table 3) and was more than twice as high in Group II as in Group I (0.692 vs. 0.329 mg/kg DM). Although the Se concentration in the feed mixture was significantly higher in Group III than in Group II, muscle Se showed only a small numerical increase, with no statistically significant difference between the two groups. This may indicate a diminishing response of muscle Se deposition to increasing dietary Se, although variation among animals and the relatively small group sizes may also have limited the detection of a difference. Furthermore, individual feed intake was not measured, so the difference in feed Se concentration

does not establish the difference in actual Se intake. These findings therefore do not confirm that muscle Se deposition had reached a plateau. Overall, the higher muscle Se concentration in Group II than in Group I, despite comparable Se concentrations in their feed mixtures, suggests that Se source played an important role in muscle Se deposition under the conditions of this study. Studies on Se-biofortified feed crops in lamb nutrition are still limited. Antunović et al. [31] evaluated Se-biofortified corn in Merinolandschaf lambs and found no significant effect on productive traits, while Se concentrations in the liver and lungs were higher in lambs receiving biofortified corn. Their study demonstrated that Se incorporated into corn through agronomic biofortification could be transferred to animal tissues, although Se speciation in muscle was not determined. The present study extends this approach by supplying Se-biofortified maize and soybean to ewes during late gestation and lactation and subsequently making the respective feed mixtures available to their lambs. It also combines individual-animal determination of total muscle Se with HPLC–ICP–MS characterization of Se species in pooled muscle material. The present findings are consistent with studies comparing conventional organic and inorganic Se supplements in lambs. Vignola et al. [11] reported that Se supplementation increased muscle Se concentration, with the greatest increase obtained with Se-enriched yeast. Se-enriched yeast increased muscle selenomethionine (SeMet), whereas sodium selenite did not produce a comparable increase in specific selenoamino acids. Juniper et al. [32] similarly reported dose-dependent increases in total Se and SeMet in most lamb tissues after supplementation with Se-enriched yeast; Se concentration in the *Psoas major* muscle was higher in lambs receiving Se-enriched yeast than in those receiving a comparable dose of sodium selenite. Paiva et al. [12] also found higher muscle Se concentrations in lambs supplemented with SeMet or Se-enriched yeast than in those receiving sodium selenite at the higher supplementation levels examined. Together, these studies support the greater capacity of organically bound Se to accumulate in skeletal muscle. The present results indicate that a similar response can be achieved when Se is supplied through agronomically biofortified feed ingredients rather than through a conventional organic Se supplement. Differences in tissue Se concentrations may also reflect the release of Se from the feed matrix during digestion, its transformation by ruminal microorganisms, and subsequent intestinal absorption. These processes may influence the amount and chemical forms of Se available for tissue deposition. Ruminal microorganisms can incorporate Se into microbial biomass or convert it into poorly available elemental Se. In an ex vivo study, SeMet resulted in greater microbial Se incorporation and less elemental Se formation than inorganic sources [33]. Such transformations may also affect Se supplied through biofortified feeds, although this was not directly assessed in our experiment. Since feed Se speciation, digestibility, and absorption were not measured, their contributions cannot be distinguished in the present study.

The feeding strategy may also have affected Se exposure in the lambs because the experimental diets were supplied to the ewes during late gestation and lactation before being consumed directly by their offspring. Stewart et al. [16] demonstrated that Se-enriched yeast administered to ewes during gestation and lactation was transferred to lambs more efficiently than sodium selenite, including greater skeletal-muscle Se concentrations in the offspring. Similarly, Jaaf et al. [34] showed that feeding Se-biofortified alfalfa hay to transition dairy cows increased Se concentrations in maternal blood and liver, colostrum Se, and blood Se in their calves. The greater muscle Se concentrations in Groups II and III may therefore have reflected both maternal transfer and the lambs' subsequent direct intake of Se-biofortified feed. Because these exposure routes were not experimentally separated, their individual contributions cannot be quantified.

The internal-organ results further indicate that Se deposition was tissue-dependent. Heart and liver Se concentrations were higher in Groups II and III than in Group I, whereas

kidney Se concentration did not differ significantly among treatments (Table 4). Nevertheless, the kidneys contained the highest total Se concentration in every group. The absence of a significant renal response, despite the high kidney concentrations, suggests that renal Se retention was less responsive to these dietary treatments than retention in muscle, heart, and liver. Juniper et al. [32] similarly reported dose-dependent increases in total Se in most tissues following supplementation with Se-enriched yeast, with the kidney being an exception to the general response. Paiva et al. [12], in contrast, found that increasing dietary Se increased serum and liver Se regardless of source, whereas the advantage of organic over inorganic Se was particularly evident in skeletal muscle. These findings emphasize that Se retention depends on both tissue type and chemical form.

4.2. Selenium Speciation in Muscle

Total Se determination was complemented by Se speciation. The HPLC–ICP–MS chromatograms indicated the presence of selenocysteine (SeCys), inorganic Se, and SeMet in pooled muscle material from all three groups (Figure 1). SeMet was the predominant quantified species and accounted for 32.4 ± 0.9%, 51.2 ± 0.7%, and 52.7 ± 0.6% of total Se in Groups I, II, and III, respectively (Figure 2). Its relative contribution was numerically higher in the pooled samples from Groups II and III than in that from Group I, whereas Groups II and III had similar values. SeCys represented only a minor fraction of total Se in all groups (Figure 2). This descriptive pattern is compatible with enhanced deposition of organically bound Se from the biofortified feed ingredients. SeMet can be incorporated non-specifically into body proteins in place of methionine and may thereby contribute to Se retention in muscle, although dietary Se metabolism and bioavailability in ruminants are also influenced by transformations in the rumen.

The observed pattern is broadly consistent with previous HPLC–ICP–MS studies of Se speciation in lamb and sheep tissues. Juniper et al. [32] observed increasing proportions of SeMet in tissues as dietary Se-enriched yeast increased, whereas comparable sodium selenite supplementation produced a substantially smaller response. Gawor et al. [35] used HPLC–ICP–MS followed by molecular mass-spectrometric confirmation to identify several Se species in lamb muscle, heart, and liver, including SeMet, selenocystine ($SeCys_2$), Se-methylselenocysteine, and inorganic Se forms. Guo et al. [22] likewise reported that SeMet was the main Se species in sheep muscle and that its proportion increased with increasing dietary SeMet or Se-enriched yeast. These studies support the biological plausibility of the numerical differences observed among the pooled samples in the present experiment, but they do not overcome the lack of independent biological replication for the present speciation data.

The analytical approach followed the broader methodological framework established by Bierla et al. [21], who developed an HPLC–ICP–MS procedure for determining SeMet and SeCys in edible animal tissues after carbamidomethylation and proteolytic extraction. Angaïts et al. [36] subsequently emphasized the importance of extraction recovery when interpreting Se speciation data. This consideration is relevant here because enzymatic digestion did not release all Se from the muscle matrix and the quantified species therefore did not account for 100% of total Se. The unquantified fraction may reflect incomplete extraction and/or additional Se species not measured by the applied procedure. The reported proportions therefore represent the Se species recovered and quantified by the method, rather than a complete speciation profile of the muscle. If extraction efficiency differed among species, their relative contributions could also be affected.

The speciation findings must therefore be interpreted conservatively. Only one pooled biological muscle sample was prepared for each group, and the three independently prepared subsamples from each pool characterized subsampling and preparation variability

rather than variability among animals. Accordingly, the numerical differences in SeMet contribution among groups (Figure 2) cannot be interpreted as statistically demonstrated treatment effects. Individual-animal total Se data showed higher muscle Se concentrations in Groups II and III than in Group I (Table 3), although dietary and pen effects could not be separated. Future studies should perform Se speciation on individual muscle samples to permit formal testing of treatment effects on the distribution of Se species.

4.3. Growth Performance and Carcass Characteristics

Despite the marked effects on tissue Se concentrations, the experimental diets did not affect lamb growth performance. Body weight increased over time, but neither dietary treatment nor the treatment × time interaction was significant, and slaughter age and average daily gain were similar among groups (Table 5). Slaughter traits were also unaffected, apart from a tendency for the combined weight of the skin and lower legs to differ among groups (Table 6). Similarly, none of the carcass measurements differed significantly, although hind leg length showed a tendency to differ (Table 7). The overall absence of effects on performance agrees with Antunović et al. [31], who found that Se-biofortified corn did not affect productive traits in fattening Merinolandschaf lambs. It is also consistent with Vignola et al. [11], Bai et al. [5], Al-Lataifeh et al. [37], and Li et al. [6], who reported limited or no effects of Se source or supplementation on growth and major slaughter or carcass traits. Se supply may already have been sufficient for normal growth in the control group, leaving little scope for further improvement. Once physiological needs are met, additional Se may increase tissue reserves without further enhancing Se-dependent functions [10]. In particular, SeMet can be incorporated into body proteins without necessarily improving growth. This offers a possible explanation for the higher tissue Se concentrations without improved performance, although baseline Se adequacy was not confirmed in the analyses presented here.

4.4. Meat-Quality Characteristics

Effects on the measured meat-quality characteristics were likewise limited. Dietary treatment did not affect pH_{45}, pH_{24}, lightness, redness, yellowness, hue angle, or chroma (Table 8). Water-holding capacity showed a tendency to decrease from Group I to Group III, but this result did not meet the predefined threshold for statistical significance and requires confirmation. Vignola et al. [11] similarly found no effects of Se source or supplementation level on pH, drip loss, cooking loss, or colour and no improvement in oxidative stability during refrigerated storage. Knowles et al. [38] also reported that SeMet markedly increased Se concentrations in lamb muscle and other tissues without appreciable effects on most meat-quality characteristics. In contrast, Bai et al. [5] found that Se-enriched yeast and selenized glucose increased muscle Se and reduced malondialdehyde concentration, while selenized glucose affected L^* and b^* after 24 h and prolonged estimated shelf life. Mariezcurrena-Berasain et al. [20] reported decreased water-holding capacity in lambs supplemented with Se-enriched yeast at 0.35 mg Se/kg DM, and Li et al. [6] observed increased redness and reduced shear force following Se supplementation. Differences among studies may reflect variation in Se source and concentration, feeding duration, background Se status, and the timing and range of meat-quality measurements. In the present experiment, colour was measured at 45 min postmortem, whereas lipid and protein oxidation and changes during refrigerated storage were not evaluated; therefore, no conclusion can be drawn regarding meat oxidative stability or shelf life. Higher tissue Se concentrations therefore indicate Se enrichment, but do not by themselves demonstrate improved antioxidant protection.

4.5. Overall Interpretation and Study Limitations

Because each dietary group was housed in a single pen and some lambs originated from the same dams and litters, potential pen- and dam-related effects could not be fully separated from dietary treatment. The findings should therefore be interpreted with these limitations in mind. Individual feed intake was not recorded, so differences in Se intake among lambs could not be quantified or accounted for when interpreting tissue Se concentrations. Because ewes and their lambs received the same dietary treatment, the findings reflect the combined maternal and lamb feeding strategy. Prenatal Se transfer, Se supply through milk, and direct feed consumption by lambs may all have contributed to the observed tissue Se concentrations. Se concentrations in maternal blood, colostrum, and milk were measured as part of the broader experiment, but these data are outside the scope of the present manuscript and are not included in the analyses reported here. The present design does not allow these contributions to be separated or the effects on lamb growth and meat quality to be attributed specifically to maternal nutrition or direct dietary intake. Overall, feeding Se-biofortified maize and soybean was associated primarily with greater Se enrichment of lamb tissues, whereas growth performance, carcass characteristics, and the basic meat-quality traits evaluated were largely unaffected. The response of Group II was particularly notable because the Se concentration of its feed mixture was comparable to that of the sodium-selenite control, while muscle Se concentration was more than twice as high (Tables 2 and 3). SeMet represented a greater numerical proportion of total Se in pooled muscle samples from Groups II and III than in Group I. This observation is consistent with differences in the forms of Se retained in muscle following consumption of Se-biofortified feeds; however, because Se speciation was performed on one pooled biological sample per group, these data are descriptive and do not permit statistical inference regarding treatment effects on individual Se species. Future studies should therefore confirm these findings using individual-animal Se-speciation analyses.

5. Conclusions

Feeding Se-biofortified maize and soybean to ewes and their lambs was associated with higher Se concentrations in lamb muscle, heart, and liver than sodium selenite supplementation, without significant differences in growth, carcass traits, or the meat-quality traits assessed. Muscle Se was higher even at a feed Se concentration comparable to the control. SeMet predominated in pooled muscle samples, but these exploratory findings require confirmation in individual animals. The results reflect the combined maternal and lamb feeding strategy, whose separate contributions could not be determined.

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