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## Proximate and mineral composition of *M. longissimus thoracis et lumborum* of suckling lambs from three Croatian indigenous breeds reared in outdoor conditions

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### ABSTRACT

Lamb meat represents an important source of nutrients for humans worldwide. However, nutritional value of lamb meat, and particularly that obtained from suckling lambs, has been scarcely investigated in comparison to other categories of meat. The study aimed to provide some basic insights into proximate and mineral composition of lamb meat and to disclose some important sources of its variability. Three Croatian indigenous insular sheep breeds were included in the study: Rab sheep, Cres sheep, and Krk. The lambs were reared in outdoor insular environment where lambs were fed exclusively by suckling their mothers and by grazing wild grown vegetation. It has been determined that gender significantly affected fat ( $p < .001$ ) and moisture ratio ( $p < .05$ ), and had no effect on concentration of the examined minerals, except Zn ( $p < .01$ ). The factor composed of breed and environment significantly affected moisture ratio ( $p < .001$ ), fat ratio ( $p < .01$ ), and concentrations of Ca, Na, Cu, and Mn ( $p < .001$ ), Zn ( $p < .01$ ), and K ( $p < .05$ ). The concentrations of Mn and Ca were by far the most influenced by breed-environment effect. The results of the study evidenced that factor composed of breed and environment represents important source of variability of nutritional value of lamb meat produced in extensive rearing systems.

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### Introduction

Ruminant meat quality traits are affected by genetic, environmental and management factors (Guerrero et al. 2013). Lamb meat falls into the category of red meat and it is considered as a valuable resource of protein, lipids and a number of micronutrients needed in the human diet (Williams 2007). Significant trend to leaner cuts of meat over the past two decades puts sheep indigenous breeds in a very favourable position in contrast to those selected for good fattening characteristics. In addition, indigenous sheep breeds have been recognised as extremely valuable for small-scale farmers worldwide due to their ability to outperform 'improved' breeds in harsh conditions. Their good adaptation to specific natural environment reflects via their firm constitution, resilience, resistance, thermo-regulation capacity, and outstanding ability to exploit various forages (Mioč et al. 2013). Their adoption for whole day walking/hiking on steep and irregular terrains in searching for food and capacity to survive

periods of food shortage by using their body reserves are particularly appreciated by breeders. These characteristics are particularly valuable for breeders whom sheep breeding represents a secondary activity. Purchase of meat is determined by the consumer habits that are largely influenced by religion, tradition and customs (Kearney 2010). Meat obtained from young lambs is particularly appreciated in numerous countries of the Mediterranean basin. Regardless of the facts that minerals play an important role in human homeostasis, and that red meat obtained from suckling lambs is an important source of these highly valuable nutrients, surprisingly little attention has been paid up to now to minerals in this category of meat. Only few scientifically based publications on this issue have been available so far (e.g. Miguélez et al. 2008; Mioč et al. 2009). The results of this study obtained on meat originating from Krk, Rab and Cres lambs raised in outdoor conditions are expected to be valuable not only for scientists involved in this topic, but also for

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producers, merchants, and consumers involved in exploitation of lamb meat.

## Material and methods

The study was conducted on carcasses that originated from suckling lambs (22 male and 14 female) of three Croatian indigenous insular sheep breeds: Rab sheep ( $n = 10$ ), Cres sheep ( $n = 12$ ), and Krk sheep ( $n = 14$ ). In order to completely mimic typical rearing system on the islands, the experimental lambs were raised in completely outdoor conditions without surveillances of the shepherds. The experimental lambs were fed only by suckling their mothers and by grazing the insular wild grown herbs. 'In situ' experiments often obstruct disentangling effects of the breed and environment, particularly when one breed is only reared in some naturally restricted area (e.g. islands, hills etc.). In order to avoid some common mistakes, these two sources of phenotypic variability were considered as one, hereinafter referred as the origin (compound factor composed of breed and environment). All procedures with the lambs were carried out in accordance with the animal welfare rules prescribed by Croatian regulations (NG 135/12:2006), and approved by the internal Ethical Committee of Faculty of Agriculture University of Zagreb. The lambs were slaughtered with  $\sim 3$  months of age ( $90 \pm 10$  days). Prior to slaughtering at a commercial abattoir, the lambs were fasted over the night with *ad libitum* access to water. After the electrical stunning and slaughtering by exsanguination, the lambs were skinned and eviscerated. The determined average hot carcass weights of Cres, Krk, and Rab lambs were  $12.92 \pm 1.42$  kg,  $9.45 \pm 0.96$  kg and  $11.00 \pm 1.56$  kg, respectively. After cooling of the carcasses at  $4^\circ\text{C}$  for 24 hours, the whole left-sided *M. longissimus thoracis et lumborum* (LTL) were taken from the carcasses. Minced, homogenised, frozen, and vacuumed samples of the LTL were sent to an accredited laboratory (Department of Chemistry and Biochemistry of Kaposvár University, Kaposvár, Hungary) for a chemical analysis. The proximate composition was determined in accordance with AOAC (Association of Official Analytical Chemists) international official methods (AOAC 2000). Nitrogen was determined by AOAC international official method 928.08 (nitrogen in meat, Kjeldahl method) and was used for calculation of nitrogen to protein content by conversion factor (6.25). Total fat was determined by AOAC international official method 991.36 [fat (crude) in meat and meat products]. Ash was determined by AOAC international official method 920.153 (ash of meat) after combustion at  $550^\circ\text{C}$  for 3 h and moisture was determined by AOAC

official method 950.46 (moisture in meat). The contents of macroelements [calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na)] and microelements [manganese (Mn), copper (Cu), zinc (Zn), and iron (Fe)] were determined by using atomic absorption spectrometry (SOLAAR M6, Thermo Electron Corporation, UK) according to European standard (EN ISO 6869:2000), whilst phosphorous (P) content was analysed by spectrometric method according to International Standard (ISO 6491:1998). Descriptive statistics and inferential statistical analysis were performed with procedures UNIVARIATE, MEANS and GLIMMIX of the SAS/STAT software, version 9.2 of the SAS system for Windows (SAS 2008) (SAS Institute Inc. Cary, NC). Since all parametric tests require normality of the data within each level of the fixed effects in the model, and homogeneity of the variances among them, the data were tested for these assumptions prior to the inferential statistical analysis. A testing for normality of the data was performed with Shapiro–Wilk's (separately for each level of the fixed effects) while the Levene's test was used for testing of the homogeneity of the variances. The majority of the data followed a normal distribution. Deviations from normality in few cases caused one or two outliers, which were removed before the final analysis. After removal of these outliers there was no more a significant evidence of non-normality of the data. In addition, removal of the outliers caused also the disappearance of all previously existing non-homogeneities of the variances. Breed–gender interaction was found to be non-significant for all investigated traits and it was not included in the statistical model in further analysis. The model (Equation 1) applied in the final statistical analysis [analysis of covariance (ANCOVA)] was as follows:

$$y_{ijk} = \beta_0 + \beta_1 x_{ijk} + O_i + G_j + e_{ijk} \quad (1)$$

for  $i = \text{Rab, Cres, Krk}$ ;  $j = \text{male, female}$  and  $k = 1, \dots, 36$ , where:  $y_{ijk}$  = observation  $k$  in level  $i$  of the breed and level  $j$  of the gender,  $\beta_0$  = the intercept,  $\beta_1$  = the regression coefficient,  $x_{ijk}$  = carcass weight  $k$  in level  $i$  of the breed and level  $j$  of the gender,  $O_i$  = the fixed effect of origin (compound factor composed of breed and environment)  $i$ ,  $G_j$  = the fixed effect of gender  $j$ , and  $e_{ijk}$  = random error. The assumptions of this model were: (i) the carcass weight was fixed and independent of the breed and gender, (ii) errors were independent of each other and (iii) errors had a normal distribution with mean 0 and variance  $\sigma^2$ . The results are presented as least square mean values with root mean square error (RMSE). *Post hoc* comparisons of the means of the minerals among the breeds were

performed with Tukey's honest significant difference test.

## Results

Estimated components of proximate composition of LTL classified by gender and origin are presented in the Table 1. The protein and ash ratios were very consistent among the genders while females had a significantly higher intramuscular fat content ( $p < .001$ ) and lower moisture content ( $p < .05$ ). The origin mostly affected the fat ratio ( $p < .01$ ) and moisture content ( $p < .001$ ) while the impacts on the protein and ash ratios were not significant. Rab and Krk lambs had a very similar proximate composition while Cres lambs had a significantly lower ratio of moisture content ( $p < .001$ ) and significantly higher ratio of fat content ( $p < .01$ ). All components of dry matter had the highest value in Cres lambs. The estimates of mineral composition of the LTL classified by the gender and origin are presented in the Table 2. Despite of being insignificant for all of the examined minerals (except for Zn,  $p < .01$ ), the effect of gender was retained in the statistical model in order to provide more credible estimates for impact of the origin. A statistically significant effect of origin on concentration of the minerals was determined for all the examined minerals except for P, Mg, and Fe. Among the examined minerals, the

concentrations of Mn and Ca were by far the most influenced by origin ( $p < .001$ ). Na was the only mineral that statistically differed among all three levels of origin. Taking into account all the examined minerals, the mineral compositions of LTL of Cres and Rab lambs considerably differed from that of Krk lambs. In comparison to Cres and Rab lambs, Krk lambs had approximately three-fold greater concentrations of Mn and Ca and almost two-fold (1.7) higher concentrations of Cu.

## Discussion

By providing lambs (carcasses) of similar age for purpose of the study, we diminished this source of phenotypic variability to a minimum, and by adjusting the marginal means for different carcass weights with the appropriate statistical model, we provided as reliable as possible estimation of the targeted effects. As it has been seen from the results, the origin (compound factor composed of breed and environment) influenced both the proximate composition and Zn content of the LTL while gender only affected proximate composition. The results regarding the proximate composition are in great accordance with some previous reports on this issue conducted under similar experimental settings (e.g. Miguélez et al. 2008; Mioč et al. 2009). However, it should be noted that the effect of the origin in above studies was reported as a

**Table 1.** Overall determined (raw means) and estimated (least square means) proximate composition (%) of meat of suckling lambs classified by gender and origin.

	Overall	Gender		Origin			RMSE	p value	
		Males	Females	Cres	Krk	Rab		Gender	Origin
Moisture	75.25	75.61	74.76	73.93 <sup>a</sup>	75.56 <sup>b</sup>	76.04 <sup>b</sup>	0.85	.011	<.001
Fat	2.51	2.13	3.08	3.29 <sup>a</sup>	2.39 <sup>b</sup>	2.12 <sup>b</sup>	0.64	<.001	.006
Protein	20.90	20.91	20.88	21.31 <sup>a</sup>	20.72 <sup>a</sup>	20.63 <sup>a</sup>	0.92	.929	.369
Ash	1.17	1.15	1.21	1.29 <sup>a</sup>	1.08 <sup>a</sup>	1.16 <sup>a</sup>	0.14	.320	.082

<sup>a,b</sup>Values within a row with different superscripts differ significantly at  $p < .05$ . RMSE: root mean square error.

**Table 2.** Overall determined (raw means) and estimated (least square means) mineral composition (mg/100g) of meat of suckling lambs classified by gender and origin.

	Overall	Gender		Origin			RMSE	p value	
		Males	Females	Cres	Krk	Rab		Gender	Origin
Ca	6.43	5.82	6.50	3.72 <sup>a</sup>	10.50 <sup>b</sup>	4.28 <sup>a</sup>	1.24	.144	<.001
P	202.48	201.32	203.91	198.05 <sup>a</sup>	206.31 <sup>a</sup>	203.50 <sup>a</sup>	8.89	.432	.313
Mg	17.25	17.16	17.48	17.17 <sup>a</sup>	17.04 <sup>a</sup>	17.78 <sup>a</sup>	0.75	.264	.088
K	313.76	314.76	310.82	295.76 <sup>a</sup>	326.14 <sup>b</sup>	316.48 <sup>b</sup>	13.89	.447	.036
Na	64.17	64.78	60.81	51.05 <sup>a</sup>	77.10 <sup>b</sup>	60.23 <sup>c</sup>	8.45	.211	<.001
Zn	1.97	2.04	1.80	1.96 <sup>ab</sup>	2.09 <sup>a</sup>	1.74 <sup>b</sup>	0.22	.006	.008
Fe	1.93	1.90	1.98	2.02 <sup>a</sup>	1.90 <sup>a</sup>	1.92 <sup>a</sup>	0.25	.399	.693
Cu	0.10	0.08	0.11	0.07 <sup>a</sup>	0.14 <sup>b</sup>	0.07 <sup>a</sup>	0.03	.006	<.001
Mn	0.02	0.02	0.02	0.01 <sup>a</sup>	0.03 <sup>b</sup>	0.01 <sup>a</sup>	0.03	.079	<.001

<sup>a,b,c</sup>Values within a row with different superscripts differ significantly at  $p < .05$ .

RMSE: root mean square error; Ca: calcium; P: phosphorous; Mg: magnesium; K: potassium; Na: sodium; Zn: zinc; Fe: iron; Cu: copper; Mn: manganese.

'pure' breed effect despite the fact that it had been confounded with the obviously present unknown environmental effects. Confounding of breed and environment is frequently present in the scientific literature when *'in situ'* experiments are conducted which is particularly pronounced when nutrition relies on wild grown vegetation. In order to reduce potential further mistakes on this issue and to avoid some serious misconceptions, we hereby appeal to proper notion of such compound factors whenever it is impossible to explicitly disentangle their components. These results represent evidence that origin represents an important source of variability of the mineral composition of lamb meat. Published results of Miguélez et al. (2008) somewhat evidence that lambs of different breeds reared in the similar environmental conditions have very similar mineral composition. The similar appearance of different breeds in the same environment, and dissimilarity between them in different environment implies that the environment (primarily nutrition) represents dominant effect in this compound factor. Results of our study pertaining to the mineral composition of the meat partially go in line with those of Miguélez et al. (2008) and Mioč et al. (2009) while considerably differ from those obtained on carcasses of fattened lambs that were slaughtered with >40 kg of body weight (Hoffman et al. 2003; El-aal and Suliman 2008). As it has been seen from the results, the meat of Cres origin had more intramuscular fat than meat of other two origins which was also accompanied with a lower concentration of almost all the examined minerals. This result is a kind of conflicting (at least partially) with some previous findings, in particular for concentrations of the Fe and Zn. More precisely, some previous researches concerning the impact of selection for lean meat yield on the nutrient content of lamb meat revealed connections between leanness of the meat and concentration of the minerals, in particular of the Fe and Zn. It has been reported that selection of animals for lean growth has been shown to increase the proportion of muscles with lower oxidative capacity (Gardner et al. 2006; Kelman et al. 2014) which have been shown to contain reduced concentrations of Fe (Pearce et al. 2009) and Zn (Kondo et al. 1991). Low appearances of Fe and Zn in the leaner meat were attributed to the factors that influence muscle fibre type and muscle oxidative capacity. These are: lower concentration of myoglobin, less vascularisation (Choi and Kim 2009; Lefaucheur 2010) and fewer mitochondria (Hoppeler 1986). By taking into account the above reports, we

can only hypothesise that these concentrations are the matter of the fibre characteristics and that fat content has probably no impact on them.

## Conclusions

The study provided few new insights on nutritional value of meat of suckling lambs of three Croatian insular breeds (known as Cres, Krk and Rab sheep) reared in outdoor production system. It was found that origin affects both the proximate composition and Zn content of lamb meat, while gender affects only the proximate composition. Aside from the disclosed impact of examined effects, the authors pointed out some of the critical points in the inferential analysis of similarly *'in situ'* conducted experiments.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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