https://doi.org/10.17221/85/2023-CJAS

# Monitoring of milk performance of Wallachian sheep grazed under traditional Carpathian management in Western Beskids location

*Martin Ptáček[1], Michal Milerski[2], Tereza Michlová[3], Jaromír Ducháček[1], Vladimír Tančin[4,5], Michal Uhrinčať[4], Jitka Schmidová[2], Filipp Georgijevič Savvulidi[1]*, Luděk Stádník[1]*

*[1]Department of Animal Science, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
*[2]Genetics and breeding of Farm Animals, Institute of Animal Science, Prague, Czech Republic*
*[3]Department of Chemistry, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic*
*[4]Research Institute for Animal Production, National Agriculture and Food Centre, Nitra, Slovak Republic*
*[5]Institute of Animal Husbandry, Faculty of Agrobiology and Food Resources, Slovak University of Agriculture in Nitra, Nitra, Slovak Republic*

**Corresponding author: savvulidi@af.czu.cz*

**Citation:** Ptáček M., Milerski M., Michlová T., Ducháček J., Tančin V., Uhrinčať M., Schmidová J., Savvulidi F.G., Stádník L. (2023): Survey of milk performance of Wallachian sheep grazed under traditional Carpathian management in Western Beskids location. Czech J. Anim. Sci., 68: 460–468.

**Abstract:** This study aimed to determine the potential of Wallachian sheep in quantitative and qualitative indicators of milk production under extensive conditions of the Western Beskids mountains. Milk samples were collected from the group of 38 non-dairying sheep selected from the basic 120-head flock. Ewes were monitored during four control days (from 27$^{th}$ April, the average 42$^{nd}$ day of lactation to 4$^{th}$ August, the average 142$^{nd}$ day of lactation) to cover the whole lactation period. Ewes were investigated for their milk production and for milk quantity parameter. Milk quality indicators included percentage estimation of milk components (fat, protein, casein, lactose, dry matter), somatic cell count ($10^3$ cells/ml), vitamin A and E content (mg/kg), and K, Mg, Ca, Zn, Cu (mg/kg) content. Milk production ranged from 1 017.37 g (early stage of lactation) to 416.87 g (late stage of lactation period). The milk contained high fat (6.06–8.44%) and protein (4.68–5.68%) percentages, low somatic cell count and favourable distribution of minerals and vitamins. In general, results of this study indicate a possibility for low-productive traditional grazing system with extensive sheep breeds not only for cultivating the area but also for the purpose of high nutritional food with beneficial aspect on human health.

**Keywords:** extensive grazing; genetic resource; mineral and vitamin content; somatic cell count

Supported by the Ministry of Agriculture of the Czech Republic (Grant QK1910156), by the Ministry of Education, Youth and Sports of the Czech Republic (Project SV23-7-21320 and SGS grant) and by the Ministry of Education, Science, Research and Sports of the Slovak Republic/the Slovak Research and Development Agency (Projects No. APVV-15-0072 and APVV-21-0134).

https://doi.org/10.17221/85/2023-CJAS

Traditional Carpathian grazing is a possibility how to cultivate extensive mountain and submountain regions using sheep. In the past, sheep farming was of great importance in the Carpathians (as well as in many other mountain areas), and Wallachian sheep were historically kept under traditional Carpathian grazing in the Western Carpathian region of Beskid Mountains (Sobala and Rahmonov 2020). According to Sobala (2018), this management plays an important role in maintaining the protected areas, keeping village identity and folk culture, and helping the tourism attractiveness of the region.

Wallachian sheep, currently kept as protected animal genetic resources in the Czech Republic, Slovakia and also *ex situ* in Germany, represent the residues of the native Zackel type sheep population in the Western Carpathians. During the 20th century mountain sheep in this region were widely crossed with foreign breeds and new populations of Improved Wallachians in former Czechoslovakia and Polish Mountain sheep were established in Poland. The original Wallachian sheep are almost extinct. The most critical period occurred in the 1980s, when only three breeding rams and a group of ewes survived. Since then, Wallachian sheep have been included in genetic resource conservation programs and their numbers have increased again. In 2022, 952 Wallachian ewes were kept in the Czech Republic (Nemecek, personal communication). Even though Wallachian sheep are not kept for dairy production any more, their historical origin in the Zackel sheep breed family suggests this possiblity. Their potential can be indicated by investigations of milk production and composition in Improved Wallachian breed in Slovakia (Oravcova et al. 2007; Oravcova 2015), Polish Mountain sheep (Kawecka et al. 2020), or Romanian Tsurcana sheep (Puie et al. 2020). Moreover, herbs growing on mountain pastures often contain specific active ingredients beneficial to health, which can become part of human nutrition through sheep milk. Milk products of Wallachian sheep grazed in mountain regions can thus become very valuable functional food. In general, information about milk performance is very scarce in this breed. Historical data were recently verified by Pesinova et al. (2011), who examined milk production and composition of original Wallachian sheep in a submountain region of Southern Bohemia. Beneficial composition of fatty acids in milk has already been confirmed in these sheep (Ptacek et al. 2019) as well as in Improved Wallachians (Meluchova et al. 2008). In sense of functional food, vitamin and mineral content in raw sheep and goat milk is in the scope of research focus (Balthazar et al. 2017). Vitamins A and E are extremely important at early stages of human life (Debier et al. 2005). Minerals, such as Ca, K, Mg, are typical of incidence and progression of cardiovascular diseases; and their antihypertensive effect has been described as well. Zn ensures the functioning of the immune system, while Cu is involved in haematopoiesis (Pereira 2014). On the contrary, high Na intake increases blood pressure. This is a practical reason for monitoring Na concentration in sheep milk used for human consumption (Trancoso et al. 2010). Moreover, vitamins and minerals usually pass from milk to cheese (Schone et al. 2003), and also influence specific properties in the process of cheese making. Michlova et al. (2014, 2016) investigated these milk attributes on dairy farms of Lacaune, East Friesian or Romanov sheep, and on dairy goat farms. Additionally, somatic cell count (SCC) has currently been reported in many studies in the context of the health status of animals and milk quality as well (Hossain et al. 2021). According to our knowledge, no information concerning SCC or mineral and vitamin content in raw Wallachian sheep milk has previously been reported yet. This shows a perspective not only for Wallachian sheep, but also for traditional Carpathian grazing as an alternative method of sheep farming. Moreover, looking for the milk potential in multiple-purpose sheep breeds used traditionally for mutton production ensures an improvement in the economics and competitiveness of smaller farms (Kuchtik et al. 2023).

The aim of this study was to evaluate milk production, milk composition, and specific milk elements in relation to food quality and human health in original Wallachian sheep naturally grazed on extensive mountain pastures.

## MATERIAL AND METHODS

All experimental procedures and handling of animals followed the rules of the Ethic Committee of the Central Commission for Animal Welfare at Ministry of Agriculture of the Czech Republic, official Czech legislation (Act No. 246/1992 Sb.) and European Directive (2010/63/EU).

https://doi.org/10.17221/85/2023-CJAS

## Farm location and flock management

The trial was conducted in a commercial flock located in the hilly area of the Western Beskid Mountains on the Czech-Polish border (Figure 1).

Sheep were kept in a traditional Carpathian grazing system with its typical aspects: free-range movement on pastures or selective grazing of montane flora. Sheep rotationally grazed individual locations highlighted in Figure 2 during the monitored period.

The stocking rate was 0.76 livestock units per hectare. No concentrate supply was available. Animals had only access to hay (*ad libitum*) as an additional supplement to compensate the potential lack of nutrition obtained from forage pasture. Breeding conditions were determined by the Western Carpathian location with the typical botanical composition of mesic Arrhenatherum grassland (dominant species *Festuca rubra, Festuca pratensis, Poa pratensis*) or Cynosurus pasture (dominant species *Cynosurus cristatus, Festuca pratensis, Trisetum flavescens*). The soil type at this region was classified as Cambisol in the pasture areas.

## Animals and milk sample collection

This study follows the trial of Ptacek et al. (2019), who aimed at fatty acid profile estimation in raw milk.

The methodical description of flock management, animals and milk sample collection was reported in this previous study. Main aspects of current monitoring were to investigate milk production, its composition, SCC, selected vitamin and mineral content throughout the lactation period. The identical group of 38 ewes (selected from the basic 120 head flock) was monitored during the entire experimental period. During the observed period three ewes lost their lambs and dried off or they dried off naturally. The number of animals varied from 38 to 32 ewes as described in the Statistical Analysis part. Ewes included in this study were selected with regard to different age (4 ewes at 1 year of age, 5 ewes at 2 years of age, 11 ewes at 3 years of age, 10 ewes at 4 years of age, 5 ewes at 5 years of age, 2 ewes at 6 years of age, 1 ewe at 7 years of age) and litter size (15 ewes with singles, 23 ewes with twins); however, lambed during the ± 15-day period. Ewes were mated with four different rams. Above all the information the nutritional status of all ewes was determined (live weight, kg; ultrasound measurements of back-muscle and back-fat thickness, mm; body condition score, BCS points).

To cover the whole lactation period ewes were monitored on four control days in approx. month intervals: 1$^{st}$ milk sampling day (Apr 27, 42$^{nd}$ day in milk on average), 2$^{nd}$ milk sampling day (May 25, 71$^{st}$ day in milk on average), 3$^{rd}$ milk sampling day (Jun 23, 100$^{th}$ day in milk on average), 4$^{th}$ milk sampling day (Aug 4, 142$^{nd}$ day in milk on average) (estimation of mineral content was reduced on three control days – 27$^{th}$ April–23$^{rd}$ June). During the experimental period, all animals were kept in one flock under identical conditions without any discernible differences in nutrition or management. All the evaluated sheep showed no signs of mastitis or other health problems. Ewes' live weight ranged from 37.75 kg to 44.6 kg with 17.8–21.8 mm back-muscle thickness and

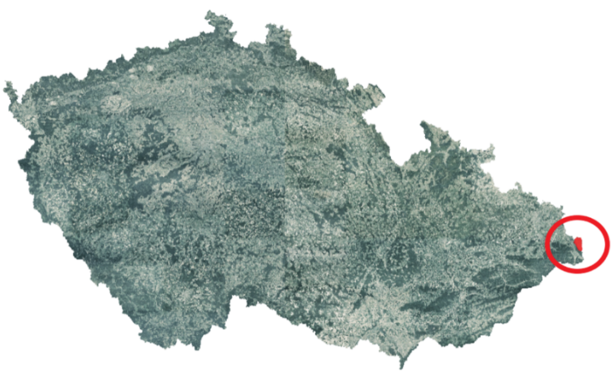

Figure 1. Location of the trial: red-coloured area in red circle

Available from https://www.mujgis.cz/nydek/index.xhtml

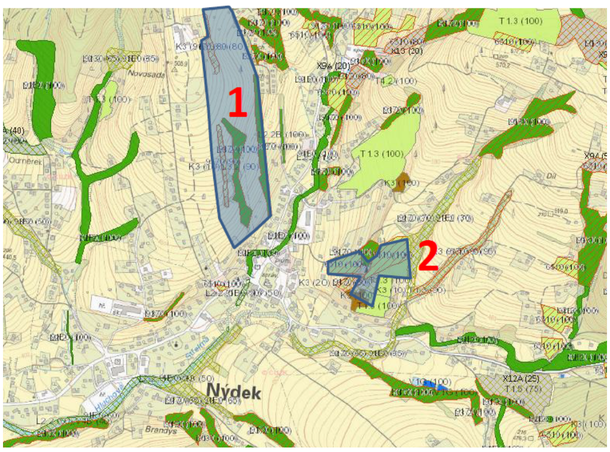


Figure 2. Mapping of pasture locations used for rotational grazing during the monitored period

https://doi.org/10.17221/85/2023-CJAS

2.7–3.8 mm back-fat thickness. The body condition scores of the observed set of animals were balanced (1.9–2.1 BCS points).

Milk sampling was done to cover the total milk production (g) including alveolar milk. After separation of their lambs the ewes were intravenously injected two international units of oxytocin for complete (cisternal and alveolar) milk ejection. Sheep were milked individually using a single bucket milking machine (regular manufacturer's setting: 38–40 kPa vacuum level and 60 : 40 pulsation ratio; InterPuls, Albinea, RE, Italy). This milk was discarded. Ewes were repeatedly injected oxytocin and milked from 317 to 501 min to achieve their total milk production. Immediately after the second milking the ewes were reunited with their lambs. The sharp time interval was recoded individually to calculate the total 24-h milk production for each ewe. Milk samples from the second milking were collected for further analyses in a standardized manner as described in protocols of the International Committee for Animal Recording.

### Milk composition and somatic cell count estimation

Milk samples for analyses of the milk components – fat, protein, casein, lactose and dry matter (%) – were added a preservative (bronopol), frozen (–20 °C), and delivered to laboratories of the Milcom a.s. Company. Milk was assayed using the Fourier transform infrared analyser operating in the central part of the infrared spectrum (Milkoscan FT2; FOSS, Hilleroed, Denmark).

Milk samples for SCC ($10^3$ cells/ml) were added a preservative (bronopol), cooled in a transport box and analysed within 24 h in a laboratory of the National Agricultural and Food Centre, Nitra). SCC were analysed on the Somacount 150 (Bentley Instruments, Chaska, MN, USA).

### Vitamin and mineral content determination

Milk samples for vitamin A and E (mg/kg), as well as for K, Mg, Ca, Zn, Cu (mg/kg) estimation were frozen (with no preservative addition), and transferred to laboratories of the Czech University of Life Sciences in Prague for these estimations.

### Analytical approach to vitamin A and E estimation

The milk sample was homogenized and 1 g of this sample was mixed with 200 µl of methanolic pyrocatechol (0.2 g/ml) and 5 ml 1 M KOH. The blend was mixed for 20 s using the vortex, saponified for 10 min in ultrasound and vortexed for 20 s again. One ml of distilled water and 5 ml of hexane were added to the mix and vortexed for 1 minute. Then 3 ml from the hexane layer were taken and evaporated until dry. The residue was dissolved in 0.5 ml of methanol and passed through a nylon filter into Eppendorf tube and put in the freezer (–20 °C) for 30 minutes. Subsequently, the sample was centrifuged for 2 min (by 14.4 rpm) and drained off into a dark vial.

The analysis was carried out using an Ultimate 3000 High Performance Liquid Chromatograph (Thermo Fisher Scientific, Dionex, Sunnyvale, CA, USA) with a quaternary pump, refrigerated autosampler, column heater and FLD and DAD detectors. Tocols and tocopherols in the sample were determined by HPLC-FLD under the following conditions: analytical column Develosil 5 µm RP AQUEOUS (250 × 4.6 mm) (Phenomenex, Torrance, CA, USA); precolumn Develosil 5 µm C30 UG-100A (10 × 4 mm) (Phenomenex, Torrance, CA, USA); mobile phase of methanol with deionised water (93 : 3, v/v), HPLC super gradient methanol (Lach-Ner, Neratovice, Czech Republic) and Milli-Q water, isocratic elution; flow rate 1 ml/min; injection 10 µl, column temperature 30 °C; FLD detection (excitation 292 nm, emission 330 nm). Retinol was determined under the same chromatographic conditions using DAD detector (λ = 325 nm). All results were expressed as mean values of three replications. The methodology used for vitamin determination is identical to a previous study of Michlova et al. (2014). Therefore, readers deeper interested in this methodical approach can find full description in this reference.

### Analytical approach to mineral estimation

The methodical description of mineral determination follows a previous stdudy of Michlova et al. (2016). Briefly, for the measurement of selected minerals approx. 300 mg of the homogenized and lyophilized sample, 2 ml $HNO_3$ and 3 ml $H_2O_2$ were added into the teflon digestion vessel. After

https://doi.org/10.17221/85/2023-CJAS

careful shaking, the mixture was allowed to stand for 30 min, then closed and microwave digested. The digest was transferred into the beaker, evaporated to wet residue, diluted with approx. 0.5 ml $HNO_3$, taken to the probes and adjusted with 1.5 $HNO_3$ to the volume of 20 ml. Concentrations of Ca, Mg, Na, K and Zn in the digest were determined by flame atomic absorption spectrometry (FAAS) using a Varian SpectrAA 110 instrument (Varian, Mulgarve, Victoria, Australia) in an acetylene-air flame at wavelengths of 422.7 nm for Ca, 285.2 nm for Mg, 589.0 nm for Na, 766.5 nm for K, and 213.9 nm for Zn. The volume of Cu was determined at the wavelength of 324.8 nm by electrothermal atomic absorption spectrometry (ETAAS) using a Varian AA 280Z (Varian, Belrose, Australia) with graphite tube atomizer GTA 120 and PSD 120 programmable sample dispenser. The background of the laboratory and used chemicals were monitored by analysis of blanks and the quality of analytical data was assessed by simultaneous analysis of certified reference material CRM 063R (Skim milk powder). Each sample was measured in triplicate, the results are expressed as mean values.

### Statistical analysis

Statistical analysis was performed using SAS v9.4. (SAS/STAT®; SAS Institute, Inc., Cary, NC, USA). The STEPWISE method in REG procedure was used to select a suitable statistical model. General linear model (GLM) with fixed effects of milk sampling day, ewe age group and litter size, and with nested effect of days in milk within particular milk sampling days was used for milk performance evaluation. The following statistical model was used for milk composition, somatic cell count, mineral and vitamin content evaluation:

$$Y_{ijkl} = DAY_i + AGE_j + LS_k + DIM_l\,(DAY) + e_{ijkl} \qquad (1)$$

where:

$Y_{ijkl}$ – measured or assessed trait (milk production, milk fat, milk protein, milk lactose, dry matter, somatic cell count, vitamin A, vitamin E, minerals: K, Mg, Ca, Zn, Cu);

$DAY_i$ – fixed effect of milk sampling day ($i$ = 1$^{st}$ milk sampling day, $n$ = 38; $i$ = 2$^{nd}$ milk sampling day, $n$ = 35; $i$ = 3$^{rd}$ milk sampling day, $n$ = 35; $i$ = 4$^{th}$ milk sampling day, $n$ = 32);

$AGE_j$ – fixed effect of ewe age group ($j$ = ewes at one year of age, $n$ = 14; $j$ = ewes at two years of age, $n$ = 20; $j$ = ewes at three years of age, $n$ = 43; $j$ = ewes at four years of age, $n$ = 34; $j$ = ewes at five years of age, $n$ = 29);

$LS_k$ – litter size ($k$ = ewes with singles, $n$ = 59; $k$ = ewes with twins, $n$ = 81);

$DIM_l$ (DAY) – nested effect of days in milk within particular milk sampling days;

$e_{ijkl}$ – residual error.

The Tukey-Kramer method was used for evaluation of differences between least square means.

## RESULTS AND DISCUSSION

The model applied to cover the variability of all the dependent variables was significant. $R^2$ values ranged from 0.580 to 0.963 for all the evaluated milk performance attributes. Significance of all factors in the statistical model is reported in Table 1.

The following parts of the manuscript are strictly aimed at the factor of the day of sample collection to demonstrate the shape of variables throughout the lactation period in Wallachian sheep. These results, summarizing all the evaluated milk performance traits, are presented in Table 2.

Milk production increased significantly from 1$^{st}$ (1 017.37 g) to 2$^{nd}$ milk sampling day (+177.94 g), and then it gradually decreased from the 3$^{rd}$ to the final level of 416.87 g milk on the 4$^{th}$ milk sampling day. All differences were supported by significant evidence among all milk sampling days. Conversely to milk production, the majority of milk components showed a significant increase as the lactation progressed. This applied to fat (from 6.06 to 8.44%), protein (from 4.68 to 5.68%), casein (from 3.47 to 4.22%), or dry matter (from 16.27 to 18.92%) percentages. On the contrary, a significantly decreased tendency throughout the monitored lactation was demonstrated for lactose percentage as the only evaluated milk component. In general, the sheep managed under extensive grazing cannot compete with the intensive dairy flock in their milk performance (Simoes et al. 2021). This was obvious in confrontation of our results with those published by Pesantez-Pacheco et al. (2019) or Robles Jimenez et al. (2020). On the contrary, milk production and milk composition of extensively kept Improved Wallachian sheep in Slovakia

Table 1. Distribution of milk components in raw milk of original Wallachian sheep over their lactation period

| Variable | $R^2$ | *P* model | DAY | AGE | LS | DAY (DIM) |
|---|---|---|---|---|---|---|
| MILK (g) | 0.950 | < 000 1 | < 000 1 | < 000 1 | 0.001 7 | < 000 1 |
| FAT (%) | 0.851 | < 000 1 | < 000 1 | 0.193 4 | 0.142 5 | 0.000 3 |
| PROT (%) | 0.865 | < 000 1 | < 000 1 | 0.710 6 | 0.894 9 | 0.009 3 |
| CAS (%) | 0.826 | < 000 1 | < 000 1 | 0.931 7 | 0.536 7 | 0.028 2 |
| LACT (%) | 0.903 | < 000 1 | < 000 1 | 0.586 4 | 0.097 2 | 0.040 2 |
| DM (%) | 0.873 | < 000 1 | < 000 1 | 0.243 0 | 0.096 9 | < 000 1 |
| SCC ($10^3$ cells/ml) | 0.963 | < 000 1 | < 000 1 | 0.644 4 | 0.054 6 | < 000 1 |
| Vitamin A (mg/kg) | 0.898 | < 000 1 | < 000 1 | < 000 1 | 0.000 4 | < 000 1 |
| Vitamin E (mg/kg) | 0.824 | < 000 1 | < 000 1 | 0.000 4 | 0.578 5 | < 000 1 |
| Na (mg/kg) | 0.723 | < 000 1 | < 000 1 | < 000 1 | < 000 1 | < 000 1 |
| K (mg/kg) | 0.714 | < 000 1 | < 000 1 | 0.076 0 | 0.105 1 | < 000 1 |
| Mg (mg/kg) | 0.630 | < 000 1 | < 000 1 | 0.548 5 | < 000 1 | < 000 1 |
| Ca (mg/kg) | 0.748 | < 000 1 | < 000 1 | < 000 1 | < 000 1 | < 000 1 |
| Zn (mg/kg) | 0.718 | < 000 1 | < 000 1 | < 000 1 | < 000 1 | < 000 1 |
| Cu (mg/kg) | 0.766 | < 000 1 | < 000 1 | < 000 1 | 0.397 6 | < 000 1 |

AGE = fixed effect of ewe age group; Ca = calcium content; CAS = milk casein; Cu = copper content; DAY = fixed effect of milk sampling day; DAY(DIM) = nested effect of days in milk within particular milk sampling days; DM = milk dry matter; FAT = milk fat; K = potassium content; LACT = milk lactose; LS = fixed effect of litter size; Mg = magnesium content; MILK = milk production; Na = sodium content; PROT = milk protein; SCC = somatic cell count; Zn = zinc content

Table 2. Distribution of milk components in raw milk of original Wallachian sheep over their lactation period

| Variable | Milk sampling day | | | |
|---|---|---|---|---|
| | Apr 27 ($n$ = 38) | May 25 ($n$ = 35) | Jun 23 ($n$ = 35) | Aug 4 ($n$ = 32) |
| MILK (g) | 1 017.37 ± 27.764$^b$ | 1 195.31 ± 28.301$^a$ | 690.59 ± 28.301$^c$ | 416.87 ± 29.938$^d$ |
| FAT (%) | 6.06 ± 0.173$^c$ | 6.81 ± 0.176$^b$ | 7.39 ± 0.176$^b$ | 8.44 ± 0.186$^a$ |
| PROT (%) | 4.68 ± 0.067$^c$ | 4.97 ± 0.068$^b$ | 5.50 ± 0.068$^a$ | 5.68 ± 0.072$^a$ |
| CAS (%) | 3.47 ± 0.068$^c$ | 3.73 ± 0.069$^b$ | 4.18 ± 0.069$^a$ | 4.22 ± 0.073$^a$ |
| LACT (%) | 5.31 ± 0.034$^a$ | 5.20 ± 0.035$^a$ | 4.88 ± 0.035$^b$ | 4.52 ± 0.037$^c$ |
| DM (%) | 16.27 ± 0.171$^c$ | 17.56 ± 0.174$^b$ | 18.11 ± 0.174$^b$ | 18.92 ± 0.184$^a$ |
| SCC ($10^3$ cells/ml) | 314.57 ± 53.677$^b$ | 399.21 ± 54.718$^b$ | 783.23 ± 55.863$^a$ | 199.30 ± 57.900$^b$ |
| Vitamin A (mg/kg) | 0.77 ± 0.022$^b$ | 1.25 ± 0.022$^a$ | 0.35 ± 0.022$^d$ | 0.49 ± 0.023$^c$ |
| Vitamin E (mg/kg) | 1.79 ± 0.104$^c$ | 3.02 ± 0.105$^b$ | 3.06 ± 0.105$^b$ | 4.78 ± 0.111$^a$ |
| Na (mg/kg) | 1 935.67 ± 29.937$^c$ | 2 227.59 ± 30.414$^a$ | 2 123.61 ± 30.414$^b$ | Data not available |
| K (mg/kg) | 9 086.52 ± 123.037$^a$ | 7 744.00 ± 124.995$^b$ | 7 079.84 ± 124.995$^c$ | Data not available |
| Mg (mg/kg) | 907.48 ± 14.655$^c$ | 951.93 ± 14.888$^b$ | 1 061.48 ± 14.888$^a$ | Data not available |
| Ca (mg/kg) | 10 490.19 ± 130.118$^a$ | 8 968.60 ± 132.189$^c$ | 9 615.75 ± 132.189$^b$ | Data not available |
| Zn (mg/kg) | 45.32 ± 0.894$^a$ | 36.67 ± 0.908$^b$ | 31.64 ± 0.908$^c$ | Data not available |
| Cu (mg/kg) | 0.64 ± 0.020$^b$ | 0.72 ± 0.020$^a$ | 0.34 ± 0.020$^c$ | Data not available |

FAT = milk fat; PROT = milk protein; CAS = milk casein; LACT = milk lactose; DM = milk dry matter; Na = sodium content; K = potassium content; Mg = magnesium content; Ca = calcium content; Zn = zinc content; Cu = copper content; Apr 27 = first milk sampling day on 27$^{th}$ of April; May 25 = second milk sampling day on 25$^{th}$ of May; Jun 23 = third milk sampling day on 23$^{rd}$ of June; Aug 4 = fourth milk sampling day on 4$^{th}$ of August

$^{a-d}$Values within a row with different superscripts differ significantly at $P < 0.05$

Data are presented as LSM ± SEM

https://doi.org/10.17221/85/2023-CJAS

(Oravcova et al. 2007) were similar to our results. Oravcova (2015) reported 0.67 kg of average daily milk production in this breed with 7.51% of fat and 5.82% of protein. Kawecka et al. (2020) informed that Polish Mountain sheep provided about 60–70 l of milk containing 8.58% of milk fat and 6.60% of milk proteins during 150 days of lactation period. The average milk production of the White (Bălă) variety of Romanian Tsurcana sheep (representative of the Zackel family of sheep phylogenetically relative to Wallachian sheep) reached 81.82 kg at 109 days long milking period (Puie et al. 2020). Our results indicate that Wallachian sheep naturally grazed under extensive conditions of the Western Carpathians achieved comparable milk production and high fat-protein content in comparison with Improved Wallachian or other phylogenetically relative sheep breeds. Additionally, the milk production of Wallachian sheep could be effectively improved even more by implementing the measurements of morphological udder characteristics as Milerski et al. (2020) suggested. There are, unfortunately, only few reports on the dairy performance of the original Wallachian sheep. Historical sources mentioned about approx. 70 l of milk produced by Wallachian sheep after lamb weaning, except of some individuals with milk yield exceeding up to 100 litres. More recently, Pesinova et al. (2011) examined milk production and composition of original Wallachian sheep in a submountain region of Southern Bohemia. They reported the average daily milk production of 0.70 l, average fat content of 4.90% and average protein content of 5.94%. Nevertheless, these results were not obtained during the regular milking period, but after separation of lambs from theirs mothers for 12 h without oxytocin application. This procedure may be a potential reason for the very low fat content in milk, as obvious from confrontation with results of this study.

SCC is a key indicator of the health status of udder and precursor to milk quality. This was confirmed by Tvarozkova et al. (2021) by strong relations between SCC and specific pathogens in raw milk of Lacaune sheep. Unfortunately, there are no standards considering SCC in raw milk of small ruminants in the EU (Kuchtik et al. 2021; Vrskova et al. 2021). In our study SCC was in a relatively wide range of 199.30 to 783.23 × $10^3$ cells/ml throughout the observed period. The highest SCC was demonstrated for the 3rd control day of milk collection, differing significantly from all other control days (this difference ranged from 384.02 to 583.93 × $10^3$ cells/ml). Additionally, non-significant differences were seen among control days 1, 2 and 4. SCC in Wallachian sheep was lower compared to those published by Leitner et al. (2016) from Israeli flocks (bulk samples) or Vrskova et al. (2021) in Slovak dairy sheep flocks. Interestingly, Vrskova et al. (2021) stated that SCC was higher in the high-productive breed Lacaune sheep and their crossbreds with East Friesian sheep compared to extensive Tsigai sheep. In the context of the results of this study it seems that dairying sheep on pastures under extensive management does not necessarily mean an increase in SCC in their milk.

Beneficial composition of fatty acids in milk has already been confirmed in original Wallachian sheep (Ptacek et al. 2019) or Improved Wallachians (Meluchova et al. 2008). This indicates that milk products of Wallachian sheep grazed in mountain regions can represent a very valuable source of food. Monitoring of vitamin and mineral content in raw sheep and goat milk is in the scope of research focus in the sense of functional food development. Some of these components have a direct positive and protective effect on human health, others have an unspecific role, which indicates the risk of specific disease occurrence. Vitamin E increased from control day 1 (1.79 mg/kg) to control day 2 (+1.23 mg/kg, $P < 0.05$), and then plateaued till the 3rd control day. Another significant increase, reaching the final level of 4.78 mg/kg, progressed from 3rd to 4th control day. A variable tendency, supported by significant differences between all control days, was detected for vitamin A. Significant decreasing tendency throughout this lactation period was monitored for K (from 9 086.52 to 7 079.84 mg/kg) and Zn (from 45.32 to 31.64 mg/kg) minerals. An opposite trend was demonstrated for Mg content (from 907.48 to 1 061.48 mg/kg). A variable tendency, however, supported by significant differences between all monitored days, was seen for Na, Ca and Cu minerals. Recio et al. (2009) stated that vitamins and minerals in sheep milk were not studied as extensively as in bovine milk, despite of their nutritional and health potential. So, it is not possible to offer a definitive opinion due to a lack of adequate research data. The majority of literature sources are identical in claiming that sheep milk is higher in vitamin and mineral content than cow's milk. Milk of Wallachian sheep

https://doi.org/10.17221/85/2023-CJAS

was mostly richer in vitamin and mineral content than the milk of unspecified sheep as published by Raynal-Ljutovac et al. (2008). Importantly, our results are within the limits detected for vitamin A and E content or minerals in specific sheep breeds. So, milk of Wallachian sheep was similar or even higher in vitamin A and E content in comparison with native Greek breeds (Kondyli et al. 2012) or in comparison with milk of East Friesian, Lacaune and Romanov sheep (Michlova et al. 2014). The same conclusion was drawn for mineral content of Wallachian sheep milk in comparison with Lacaune, East Friesian and Romanov sheep (Michlova et al. 2016). In this sense it can be concluded that Wallachian sheep milk is also a valuable source of important vitamins and minerals for consumers.

## CONCLUSION

Results of this study indicate the potential for low-productive traditional grazing system with extensive sheep breeds not only for cultivating the area but also for achieving valuable functional food with beneficial aspects in human nutrition. Additionally, the use of Wallachian sheep for dairy production would also be helpful to their more secure preservation as a genetic resource.

## Conflict of interest

The authors declare no conflict of interst.

## REFERENCES

Balthazar CF, Pimentel TC, Ferrao LL, Almada CN, Santillo A, Albenzio M, Mollakhalili N, Mortazavian AM, Nascimento JS, Silva MC, Freitas MQ, Sant'Ana AS, Granato D, Cruz AG. Sheep milk: Physicochemical characteristics and relevance for functional food development. Comprehen Rev Food Sci Food Saf. 2017 Jan;16(2):247-62.

Debier C, Pottier J, Goffe C, Larondelle Y. Present knowledge and unexpected behaviours of vitamins A and E in colostrum and milk. Livest Prod Sci. 2005 Dec; 98(1-2):135-47.

Hossain D, Singha S, van Thof CM, Gough S, Uddin AM. Effect of somatic cells on milk quality and human health. Vet Sci Res Rev. 2021 Feb;7(1):31-4.

Kawecka A, Pasternak M, Sloniewska D, Miksza-Cybulska A, Bagnicka E. Quality of mountain sheep milk used for the production of traditional cheeses. Annals Anim Sci. 2020 Jan;20(1):299-314.

Kondyli E, Svarnas C, Samelis J, Katsiari MC. Chemical composition and microbiological quality of ewe and goat milk of native Greek breeds. Small Rumin Res. 2012 Apr;103(2-3):194-9.

Kuchtik J, Sustova K, Sykora V, Kalhotka L, Pavlata L, Konecna L. Changes in the somatic cells counts and total bacterial counts in raw goat milk during lactation and their relationships to selected milk traits. Ital J Anim Sci. 2021 Jun;20(1):911-7.

Kuchtik J, Sustova K, Kopec T, Pajor F, Konecna L. Experimental evaluation of milk yield and selected traits of milk quality in Clun Forest ewes. Czech J Anim Sci. 2023 Jul; 68(7):306-12.

Leitner G, Lavon Y, Matzrafi Z, Benun O, Bezman D, Merin U. Somatic cell counts, chemical composition and coagulation properties of goat and sheep bulk tank milk. Int Dairy J. 2016 Jul;58:9-13.

Meluchova B, Blasko J, Kubinec R, Gorova R, Dubravska J, Margetin M, Sojak L. Seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. Small Rumin Res. 2008 Aug;78(1-3):56-65.

Michlova T, Hornickova S, Dragounova H, Hejtmankova A. Quantitation of vitamins A and E in raw sheep milk during lactation period. Agron Res. 2014;12(3):737-44.

Michlova T, Hejtmankova A, Dragounova H, Hornickova S. The content of minerals in milk of small ruminants. Agron Res. 2016;14(S2):1407-18.

Milerski M, Ptacek M, Duchacek J, Schmidova J, Uhrincat M, Stadnik L, Tancin V. Analysis of the relationship between milk production, milk composition and morphological udder measurements in Wallachian sheep. Czech J Anim Sci. 2020 Nov;65(11):424-30.

Oravcova M. The effect of stage of lactation on daily milk yield, and milk fat and protein content in Tsigai and Improved Valachian ewes. Mljekarstvo. 2015 Jan;65(1):48-56.

Oravcova M, Margetin M, Peskovicova D, Dano J, Milerski M, Hetenyi L, Polak P. Factors affecting ewe's milk fat and protein content and relationships between milk yield and milk components. Czech J Anim Sci. 2007 Jul; 52(7):189-98.

Pereira PC. Milk nutritional composition and its role in human health. Nutrition. 2014 Jun;30(6):619-27.

Pesantez-Pacheco JL, Heras-Molina A, Torres-Rovira L, Sanz-Fernandez MV, Garcia-Contreras C, Vazquez-Gomez M, Feyjoo P, Caceres E, Frias-Mateo M, Hernandez F, Martinez-Ros P, Gonzalez-Martin JV, Gonzalez-Bulnes A, Astiz S. Maternal metabolic demands caused by pregnancy and lactation: Association with productivity and offspring phenotype in high-yielding dairy ewes. Animals. 2019 May; 9(6): 19 p.

Pesinova P, Vejcik A, Marsalek M. Milk quality of the Original Valachian in a submontane region. J Agrobiol. 2011 Jan;28(2):147-55.

Ptacek M, Milerski M, Duchacek J, Schmidova J, Tancin V, Uhrincat M, Stadnik L, Michlova T. Analysis of fatty acid profile in milk fat of Wallachian sheep during lactation. J Dairy Res. 2019 Apr;86(2):233-7.

Puie A, Raducuta I, Poliopola A, Marmandiu A, Calin I. Research on performances in the production of milk carried out by the Bala ecotype of the Tsurcana breed. Sci Papers Ser D Anim Sci. 2020;63(1):366-72.

Raynal-Ljutovac K, Lagriffoul G, Paccard P, Guillet I, Chilliard Y. Composition of goat and sheep milk products: An update. Small Rumin Res. 2008 Sep;79(1):57-72.

Recio I, de la Fuente MA, Juarez M, Ramos M. Bioactive components in sheep milk. In: Park YW, editor. Bioactive components in milk and dairy products. New Jersey, USA: Wiley-Blackwell; 2009. p. 83-104.

Robles Jimenez LE, Angeles Hernandez JC, Palacios C, Abecia JA, Naranjo A, Osorio Avalos J, Gonzalez-Ronquillo M. Milk production of Lacaune sheep with different degrees of crossing with Manchega sheep in a commercial flock in Spain. Animals. 2020 Mar 20;10(3): 10 p.

Schone F, Leiterer M, Hartung H, Jahreis G. Trace elements and further nutrition-related constituents of milk and cheese. Milchwissenschaft. 2003 Sep;58(9-10):486-90.

Simoes J, Abecia JA, Cannas A, Delgadillo JA, Lacasta D, Voigt K, Chemineau P. Review: Managing sheep and goats for sustainable high yield production. Animal. 2021 Dec;15(S1): 12 p.

Sobala M. Pasture landscape durability in the Beskid Mountains (Western Carpathians, Poland). Geographia Polonica. 2018 Feb;91(2):197-216.

Sobala M, Rahmonov O. The human impact on changes in the forest range of the Silesian Beskids (Western Carpathians). Resources. 2020 Dec;9(12): 20 p.

Trancoso IM, Trancoso MA, Martins APL, Roseiro LB. Chemical composition and mineral content of goat milk from four indigenous Portuguese breeds in relation to one foreign breed. Int J Dairy Technol. 2010 Aug;63(4):516-22.

Tvarozkova K, Vasicek J, Uhrincat M, Macuhova L, Hleba L, Tancin V. The presence of pathogens in milk of ewes in relation to the somatic cell count and subpopulations of leukocytes. Czech J Anim Sci. 2021 Jul;66(8):315-22.

Vrskova M, Tancin V, Uhrincat M, Macuhova L, Tvarozkova K. Survey of somatic cell counts and milk composition in bulk milk of ewes in dairy practice. Slovak J Food Sci. 2021 Mar;15:151-5.

Received: June 17, 2023
Accepted: October 12, 2023
Published online: November 15, 2023