Analysis of fatty acid profile in milk fat of Wallachian sheep during lactation

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SUPPLEMENTARY FILE

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Material and Methods

Flock location and management

The study was performed in one flock, located in Beskydy Mountain, Moravian-Silesian region at the altitude 450 – 890 m a.s.l. (above sea level). The local annual rainfall was 833 mm and the average annual temperature was 7.0°C in the evaluated year. The sheep were naturally grazed on the permanent pasture under traditional Carpathian management system, lying in free motion on pastures, selective grazing montane region of Beskydy Mountains, and no concentrates supply. Ewes had access to hay (*ad libitum*) as additional supplement to compensate potential lack of nutrition obtained from forage pasture. Pastures can be characterized as 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 was classified as Cambisol in the pasture areas. The stocking rate was 0.76 livestock units per hectare. The lambing occurred from the beginning of March to mid. April on pastures. After lambing all lambs were identified to their mothers, weighted and tagged. The lambs were kept together with their

mothers up to fifth month of age (end of July), when the ram lambs were separated from the flock. Ewe lambs were kept with their mothers up to mating period.

FAs profile estimation

The gravimetric method was performed for milk fat extraction (ČSN EN ISO 1211 (570534)). The milk fat extract (60 mg) was obtained using a water-based-solution of ammonia, ethanol, diethyl ether and petroleum ether. FA methyl esters were prepared by the potassium hydroxide catalysed methylation and extracted into hexane. Gas chromatography (GC, FID) of FA methyl esters was performed using the Master GC (DANI Instruments S.p.A.; Italy) (split regime, FID detector) on a column with polyethylene glycol stationary phase (FameWax – 30 m × 0.32 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 5 ml × min^{-1}. The temperature programme used for GC was as follows: 50 °C (2 min), after which the temperature was increased to 230 °C at 10 °C × min (8 min), the temperature of the detector being 220 °C.

Statistical evaluation

The following statistical model was used for all FAs or FA groups evaluation:

$$Y_{ijklm} = DAY_i + AGE_j + DIM_k\,(DAY) + an_l + e_{ijklm}$$

Where:

Y_{ijklm} = measured or assessed trait;

DAY_i = fixed effect of control day of milk collection (i = 1st day of milk collection, n = 38; i = 2nd day of milk collection, n = 35; i = 3rd day of milk collection, n = 35; i = 4th day of milk collection, n = 32);

50 AGE_j = fixed effect of ewe age category (j = 1 year old ewes, n = 14; j = 2 years old ewes, n =
51 20; j = 3 years old ewes, n = 44; j = 4 years old ewes, n = 33; j = 5 years and older ewes, n =
52 29);
53 DIM_k (DAY) = nested effect of days in milk within particular control days of milk collection;
54 an_l = animal (random effect);
55 e_{ijklm} = residual error.
56 The Tukey-Kramer method was used for evaluation of differences between least square
57 means. Significance level $P < 0.05$ was used to evaluate the differences among groups.
58
59 **References**
60 **ISO EN 2010 1211 ČSN 570534** 2010 Milk–Determination of fat content-Gravimetric
61 method (Reference method). *European standard EN ISO* 1211
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Table S1. Basic characteristic of Wallachian sheep milk composition

Variable	N	AM	SD	MIN	MAX	V (%)
Milk production (kg)	140	0.85	0.41	0.14	2.60	48.33
Milk protein (%)	140	5.18	0.61	3.85	7.49	11.74
Milk lactose (%)	140	5.01	0.36	3.75	5.85	7.23
Milk fat (%)	140	6.98	1.49	2.71	13.30	21.33

Supplementary file Table S1 legend.

N = number of observing; AM = arithmetic mean; SD = standard deviation; MIN = minimal value; MAX = maximal value; V = variation coefficient.

Table S2. Basic database structure of fatty acids profile in milk fat of Wallachian sheep

Variable	N	AM	SD	MIN	MAX	V (%)
C4:0	140	1.81	0.28	0.94	2.50	15.46
C6:0	140	1.52	0.33	0.83	2.41	21.80
C8:0	140	1.46	0.43	0.64	2.51	29.76
C10:0	140	4.20	1.60	1.49	9.06	38.21
C11:0	140	0.16	0.04	0.04	0.54	47.63
C12:0	140	2.64	0.99	1.15	7.94	37.38
C14:0R	140	0.16	0.04	0.08	0.27	23.48
C14:0	140	7.43	2.25	3.74	16.74	30.26
C15:0R	140	0.41	0.07	0.20	0.58	17.54
C14:1	140	0.11	0.08	0.02	0.56	73.72
C15:0	140	1.15	0.19	0.06	1.69	16.52
C16:0R	140	0.36	0.08	0.18	0.94	22.01
C16:0	140	20.04	2.91	16.49	27.87	12.08
C16:1T	140	0.65	0.09	0.42	0.88	14.36
C16:1	140	0.84	0.22	0.39	1.72	26.43
C17:0	140	0.84	0.13	0.54	1.20	14.66
C17:1	140	0.32	1.09	0.19	0.47	17.79
C18:0	140	15.05	3.12	4.92	22.39	19.09
∑C18:1elaivak	140	7.90	2.92	0.52	15.23	31.59
C18:1n9c	140	22.00	5.10	12.42	33.33	21.94
∑C18:1C	140	2.03	0.27	1.16	3.37	13.51
∑C18:2T	140	1.79	0.30	0.85	2.83	16.91
C18:2n6c	140	1.81	0.41	1.02	2.88	21.66
C20:0	140	0.28	0.05	0.14	0.41	17.21
C18:3n6	140	0.08	0.02	0.04	0.15	25.83
C21:0	140	0.10	0.22	0.01	2.00	169.88
C22:0	140	0.16	0.03	0.04	0.27	19.79
C20:4n6	140	0.12	0.03	0.05	0.19	24.60
C20:5n3	140	0.11	0.02	0.08	0.16	16.00
CLA	140	2.19	0.80	0.13	4.41	35.84
SFA	140	57.99	5.86	45.18	75.53	8.89
MUFA	140	34.05	5.78	18.90	46.23	14.20
PUFA	140	7.95	1.09	5.57	10.56	12.85

Supplementary file Table S2 legend.

N = number of observing; AM = arithmetic mean; SD = standard deviation; MIN = minimal value; MAX = maximal value; V = variation coefficient; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids.