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Original study

The effect of cattle breed, season and type of diet on nitrogen fractions and amino acid profile of raw milk

Ludmila Křížová[1,2], Oto Hanuš[1], Petr Roubal[3], Josef Kučera[1,4] and Sylvie Hadrová[2]

[1]Research Institute for Cattle Breeding, Ltd., Rapotín, Czech Republic, [2]Agrovyzkum Rapotín Ltd., Rapotín, Czech Republic, [3]Dairy Research Institute Ltd., Praha, Czech Republic, [4]Faculty of Agronomy, Mendel University in Brno, Czech Republic

Abstract

The aim of the study was to describe the differences in nitrogen fractions and deepen the knowledge in the amino acid profile of raw milk affected by the breed of cattle, season and type of feeding. The study was conducted from June 2005 to February 2007 on 64 bulk milk samples collected from eight herds consisting of Czech Fleckvieh (four herds) and Holstein (four herds) breed. One half of the herds of each breed was grazed while the other half was not. Samples were collected twice in winter and twice in summer. The effect of the breed resulted in differences in milk yield that was lower in Czech Fleckvieh (5 385.50 kg) than in Holstein (7 015.15 kg, $P<0.05$). The content of nitrogen fractions was higher in Czech Fleckvieh than in Holstein ($P<0.05$). No effect of the breed on the amino acid profile of milk was observed except on the concentration of Glu ($P<0.05$). The effect of the season was demonstrated in the decrease of the concentrations of nitrogen fractions and Met during summer in comparison to winter ($P<0.05$). The effect of the type of feeding resulted in lower milk yield (5 197.50 and 7 203.75 kg) and lower concentrations of nitrogen fractions in grazed herds compared to non-grazed herds ($P<0.05$), respectively. Furthermore, the amino acid profile of milk differed significantly between grazed and non-grazed herds ($P<0.05$).

Keywords: dairy cow, Czech Fleckvieh, Holstein, bulk milk, pasture, season

Abbreviations: CAS: casein, CP: cude protein, NPN: non protein nitrogen, TMR: total mixed ration, TP: true protein, WP: whey proteins

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Corresponding author:
Ludmila Krizova; email: ludmila.krizova@vuchs.cz
Research Institute for Cattle Breeding Ltd., Department of animal nutrition and quality of livestock products, Vyzkumniku 267, Vikyrovice, 78813, Czech Republic

Introduction

In recent years, the demand for food with high nutrient value and high sensory quality has increased. High quality milk is important for the production of high quality pasteurised market milk and dairy products (Elmoslemany *et al.* 2010). Thus the knowledge of the composition of raw cows' milk is of the greatest importance not only for the dairy industry but also for consumers. Not only the economic impact of variation in fat and protein content has led to much research on manipulation and alteration of these constituents, but also the different amino acid, fatty acid, micronutrient profiles and their consequent impact on health have become current topics of debate. Increased knowledge of the impact of feeding on the quality and quantity of milk production has led to more sophisticated diet formulations for cattle (Schönfeldt *et al.* 2012, Jenkins & McGuire 2006). Thus, the determination of the AA profile in milk is essential for qualitative evaluation of peptides and proteins that can affect the chemical and nutritional properties of milk (Marino *et al.* 2010).

The nitrogen fractions of milk can be broadly divided into three categories; casein (CAS), whey proteins (WP) and non protein nitrogen (NPN). Casein comprises the majority of nitrogen in milk (about 78 %) and is a determinant of the technological usability of milk while WP (17 %) are an indicator of the positive health value of milk for humans (Litwińczuk *et al.* 2011). The smallest fraction, NPN, represents 5 % of the nitrogen in milk (Jenkins & McGuire 2006). The variation in nitrogen fractions of milk affects consistency, yield and quality of dairy products (Mehra *et al.* 1999). Although genetics, in particular milk protein polymorphisms, play a major role in regulating milk protein composition (Bobe *et al.* 1999, Graml & Pirchner 2003) less conclusive effects of other factors such as breed (*e.g.* Chrenek *et al.* 1987), season (*e.g.* Lindmark-Månsson *et al.* 2003, Kriščiunaite *et al.* 2012), nutrition (*e.g.* Hadrová *et al.* 2007, Cant *et al.* 2003, Bargo *et al.* 2002) or animal health (*e.g.* Csapó *et al.* 1995) have been documented in literature. Therefore, altering milk protein composition by selective breeding or nutrition could be useful for the dairy industry (Bobe *et al.* 2009).

The aim of the study was to determine the effect of cow breed, season and type of diet on the content of nitrogen fractions and amino acid profiles of raw milk.

Material and methods

The study was conducted from June 2005 to February 2007 on bulk milk samples collected from eight commercial dairy herds consisting of Czech Fleckvieh (four herds) and Holstein (four herds) dairy cows. The average herd size was 185 ± 149 (from 66 to 439) dairy cows and the average milk yield was 6 200.6 ± 1 455.1 (from 3 836 to 8 124) kg (see Table 1 also for details about selected farms environmental characteristics). Cows were fed diets typical in the Czech Republic which consist of maize silages, clover-grass haylages, hay, locally available feedstuffs, concentrate and mineral mixtures according to relevant milk yield and standard requirements. One half of the herds of each breed was grazed during summer season while the other half was not as given in Table 2. Cows were milked twice a day and samples were collected regularly two times in the winter and two times in the summer period resulting in a total of 64 bulk milk samples examined.

Table 1
Basic information about environment of dairy cow herds

Farm	Breed	n	Milk yield/ lactation, kg	Altitude, m	Annual rainfall, mm	Mean annual T, °C
1	CF	315	6445	440	650	7.90
2	CF	68	6735	360	700	7.00
7	CF	73	4526	550	900	4.50
8	CF	75	3836	680	1140	7.40
	x	132.8	5385.5	507.5	847.5	6.70
	SD	121.5	1424.0	138.9	222.9	1.52
3	H	125	6790	250	700	7.80
4	H	66	5638	520	720	4.80
5	H	439	7511	390	650	5.50
6	H	318	8124	286	670	9.60
	x	237	7015.8	361.5	685.0	6.90
	SD	172.4	1068.1	121.2	31.1	2.20
Total	x	184.9	6200.6	434.5	766.3	6.80
	SD	148.9	1455.1	143.7	171.0	1.75

CF: Czech Fleckvieh; H: Holstein, n: number of cows per herd, x: mean, SD: standard deviation

Table 2
Composition of diets (kg/d, as fed basis) of dairy cows used in experimental herds

		Summer diet							Winter diet				Year-round diet					
Farm	n	PAS	HAY	HAYL	SIL	SM	MM	BD	GPS	SIL	HAYL	BS	HAY	MM	BD	MOL	SM	SCH
1	315								4	10	9		1.5	0.2	7		1.5	
2	68									17	19		1.5	0.1			4.5	2.0
7	73	50	3.0			2.0	0.10		40				5.0	0.1			2.5	
8	75	50	2.5			2.5	0.15	5			35		5.0	0.2	2	0.6	2.5	
3	125	25		12	10	4.0	0.15		22		15	0.7		0.8			4.7	0.9
4	66	40		5	5	0.5	0.10				25		3.0	0.1	12		0.5	
5	439								20		14		0.7	0.2			2.6	1.4
6	318									17	13	0.5	0.5	0.5			6.0	7.0

n: number of cows per herd, PAS: grass and herbal pasture, HAY: meadow hay, HAYL: clover-grass haylage, SIL: maize silage, SM: supplemental mixture, MM: mineral mixture, BD: brewer's draff, GPS: silage from whole maize plant in vax maturity, BS: barley straw, SCH: sugarbeet chippings, MOL: molasses

All bulk milk samples were transported to the accredited National Reference Laboratory for raw milk (according to ČSN EN ISO/IEC 17025) in the Research Institute for Cattle Breeding, Ltd. in Rapotin (Czech Republic) for analyses. Data concerning daily milk performance were obtained from animal records kept from milk recording kept by the Czech Moravia Breeders Corporation.

Analyses

The crude and true protein and casein (CP, TP and CAS) were determined by reference Kjeldahl's method using the instrument line Tecator with Kjeltec Auto Distillation unit 2 200 (Foss-Tecator AB, Höganäs, Sweden) according to ČSN 57 0530. The instrument was included in international proficiency testing (APLAC and ICAR–CECALAIT) with regularly mostly successful results.

Milk amino acid profile was determined as described in Hadrová *et al.* (2007). Briefly, samples of lyophilised milk for acid hydrolysis were hydrolysed with 6 mol/L HCl for 24 h at 110 °C. Sulphur amino acids were determined as cysteic acid and methionine-sulphone. All hydrolysates were separated in the automatic amino analyser AAA 400 (Ingos, Prague, Czech Republic) using Na citrate buffer system and quantified by reaction with ninhydrin. For determination of the amino acid content the programme ChromuLan v. 0.7 (http://www.chromulan.org) was used. There were investigated 17 amino acids in the samples. The results were expressed absolutely in g/kg and relatively in %.

Statistical analysis

The GLM procedure of the SAS v. 9 programme package (SAS Institute Inc., Cary, NC, USA) was used for the calculation. Multi-factor analysis of variance with fixed effects as breed, season and feeding was used for statistical evaluation of data set according to following model:

$$y_{ijk}=\mu+b_i+s_j+f_k+e_{ijk} \tag{1}$$

where y_{ijk} is the independent variable (investigated milk indicator), μ is the general mean, b_i is the effect of breed (i=2), s_j is the effect of season (j=2), f_k is the effect of feeding (k=2), and e_{ijk} is the random effect.

Results and discussion

The composition of raw milk, especially the content of nitrogen fractions determines, to a large extent, the yield, the nutritional value and technological properties of a number of dairy products (Heck *et al.* 2009, Mehra *et al.* 1999). Thus, variations in milk composition are of a great importance to the manufacturers of dairy products. Recently, several studies have shown a large variation in the relative concentration of the major milk proteins (Bobe *et al.* 1998, Bordin *et al.* 2001, Heck *et al.* 2008) but it has been found that a considerable part of this variation is genetic (Bobe *et al.* 1999, Graml & Pirchner 2003). The role of nutrition on milk protein composition is also documented in the literature (recently *e. g.* Bobe *et al.* 2009, Hadrová *et al.* 2007, Doepel *et al.* 2004), however results of these studies are inconsistent and the role of nutrition on manipulating protein composition of milk is less conclusive (Bobe *et al.* 2009).

Milk contains a mixture of proteins, each having unique attributes for nutritional, biological and human food ingredient applications (Smithers *et al.* 1996). The role of milk and dairy products in human nutrition and their impact on consumers' health is one that has been hotly debated by both the food and health industries. However, this discussion often fails to mention the fact that dairy products are among the most versatile protein sources available with a unique amino acid composition (Dunshea *et al.* 2007). Although milk protein polymorphisms play a major role in regulating the milk protein composition as documented by *e. g.* Graml & Pirchner (2003), in a recent study Lindmark-Månsson *et al.* (2003) reported that the amino acid profile of milk not only differs among countries, but also within the same country and between seasons, probably due to feeding changes. From this point of view knowledge concerning not only content and proportion of nitrogen fractions but also amino acid composition of milk is valuable for the assessment of milk quality and its nutritional and manufacturing value (Guy & Fenaille 2006).

To our knowledge, only a very limited number of studies have analysed the amino acid profile of raw milk and the concentration of nitrogen fractions in relation to breed, season and type of feeding.

Effect of the breed

Milk yield and concentrations of nitrogen fractions in dependence on breed, season and type of feeding are presented in Table 3. Milk yield in Czech Fleckvieh breed was 5 385.50 kg and was lower than the yield determined in Holstein breed (7 015.15 kg, $P<0.05$). Results obtained in our study are in agreement with data describing milk yield of these two breeds in the Czech Republic environment (*e. g.* Wolfová *et al.* 2007, Hanuš *et al.* 2010). There were several significant differences in the content of nitrogen fractions between the two breeds. Czech Fleckviehs produced significantly ($P<0.05$) higher concentrations of CP, CAS, TP and NPN than Holsteins. The content of WP did not differ significantly between breeds. Similar findings were reported by *e. g.* Hanuš *et al.* (2010) and Wolfová *et al.* (2007). Furthermore, values determined in Holstein are comparable with the data reported in the literature such as Lindmark-Månsson *et al.* (2003), Kriščuinate *et al.* (2012), Jõudu *et al.* (2008) or Wedholm *et al.* (2006) for this breed.

Table 3
Milk yield and protein fractions as affected by the breed, season and type of feeding

	Czech Fleckvieh	Holstein		Summer	Winter		Grazed herds	Non-grazed herds		Breed	Season	Feeding
	Mean	Mean	SEM	Mean	Mean	SEM	Mean	Mean	SEM	*P*	*P*	*P*
Milk yield	5 385.50	7 015.75	77.887	6 200.63	6 200.63	77.887	5 197.50	7 203.75	77.887	<.0001	1.0000	<.0001
Crude protein	3.37	3.25	0.018	3.27	3.34	0.018	3.22	3.39	0.018	<.0001	0.0032	<.0001
Casein	2.66	2.58	0.017	2.59	2.66	0.017	2.56	2.68	0.017	0.0014	0.0022	<.0001
True protein	3.16	3.07	0.014	3.06	3.17	0.014	3.04	3.19	0.014	<.0001	<.0001	<.0001
Whey protein	0.50	0.49	0.011	0.48	0.51	0.011	0.47	0.51	0.011	0.5653	0.0394	0.0242
Non protein nitrogen	0.21	0.18	0.010	0.20	0.18	0.010	0.18	0.20	0.010	0.0331	0.0368	0.2848

The concentration (g/kg) of amino acids in milk in dependence on cattle breed, season and type of diet are given in Table 4. The total content of amino acids in milk in Czech Fleckvieh was 30.868 g/kg and in Holstein 30.078 g/kg and it was not affected by the cattle breed (P>0.05). The concentration of essential amino acids and non-essential amino acids in milk was similar in both breeds and did not differ significantly (P>0.05). The tendency was found for the total content of semi-essential amino acids (Cys+Tyr), that it was higher in Czech Fleckvieh, being 1.670 g/kg, than in Holstein (1.596 g/kg, P=0.0971). From all individual amino acids the highest concentration of Glu was found in both breeds followed by Pro, Leu and Lys. For the individual essential amino acids no effect of the breed was observed except for the concentration of Glu that was higher in Czech Fleckvieh in comparison to Holstein (P<0.05) and for Tyr that tended to be higher in Czech Fleckvieh than in Holstein (P=0.083). Our results are comparable with the values published in the literature (Schönfeldt *et al.* 2012, Lindmark-Månsson *et al.* 2003, Swaisgood 1995, Davis *et al.* 1994) confirming the genetical background of amino acid composition of milk proteins with very small breed differences. However, minor differences in the amino acid profile of milk between two cattle breeds and their crosses were mentioned by Chrenek *et al.* (1987) or Mapekula *et al.* (2011).

Table 4
Effect of cattle breed, season and type of feeding on amino acid profile (g/kg) of milk protein

Amino acid	Czech Fleckvieh	Holstein		Summer	Winter		Grazed herds	Non-grazed herds		Breed	Season	Feeding
	Mean	Mean	SEM	Mean	Mean	SEM	Mean	Mean	SEM	*P*	*P*	*P*
Ala	0.853	0.852	0.013	0.869	0.836	0.013	0.827	0.877	0.0125	0.9405	0.0652	0.0065
Arg	1.245	1.227	0.016	1.226	1.245	0.016	1.209	1.263	0.0164	0.4353	0.4214	0.0226
Asp	2.335	2.262	0.038	2.260	2.337	0.037	2.238	2.359	0.0372	0.1665	0.1484	0.0257
Cys	0.190	0.179	0.009	0.182	0.187	0.009	0.173	0.196	0.0090	0.3720	0.6641	0.0818
Glu	6.020	5.834	0.053	5.899	5.955	0.053	5.796	6.059	0.0534	0.0171	0.4587	0.0009
Gly	0.567	0.555	0.009	0.557	0.565	0.009	0.553	0.569	0.0088	0.3244	0.4958	0.2161
His	0.860	0.857	0.010	0.856	0.861	0.010	0.836	0.881	0.0101	0.8152	0.7175	0.0028
Ile	1.517	1.475	0.026	1.486	1.507	0.026	1.455	1.538	0.0256	0.2326	0.5567	0.0252
Leu	2.917	2.857	0.038	2.896	2.878	0.038	2.810	2.964	0.0381	0.2625	0.7277	0.0057
Lys	2.509	2.443	0.034	2.454	2.498	0.034	2.396	2.555	0.0343	0.1738	0.3593	0.0020
Met	0.985	0.939	0.043	0.887	1.038	0.043	0.962	0.962	0.0427	0.4554	0.0151	0.9914
Phe	1.466	1.423	0.019	1.421	1.468	0.019	1.409	1.480	0.0189	0.1188	0.0827	0.0106
Pro	3.172	3.112	0.078	3.223	3.061	0.078	3.053	3.232	0.0780	0.5891	0.1469	0.1103
Ser	1.600	1.572	0.025	1.584	1.587	0.025	1.546	1.626	0.0254	0.4355	0.9461	0.0300
Thr	1.269	1.238	0.018	1.235	1.273	0.018	1.215	1.293	0.0183	0.2331	0.1408	0.0042
Tyr	1.480	1.417	0.025	1.425	1.473	0.025	1.404	1.493	0.0250	0.0830	0.1789	0.0142
Val	1.880	1.833	0.026	1.844	1.869	0.026	1.806	1.907	0.0255	0.1923	0.4882	0.0067
Total AA	30.868	30.074	0.395	30.303	30.639	0.395	29.690	31.252	0.3950	0.1603	0.5497	0.0069
EAA	13.406	13.065	0.191	13.079	13.393	0.191	12.892	13.580	0.1912	0.2128	0.2506	0.0135
SEAA	1.670	1.596	0.031	1.606	1.659	0.031	1.577	1.688	0.0311	0.0971	0.2287	0.0137
NEAA	15.792	15.413	0.182	15.618	15.587	0.182	15.222	15.983	0.1825	0.1471	0.9031	0.0045

AA: amino Acid, EAA: essential AA (Thr+Val+Met+Ile+Leu+Phe+Lys+His), SEAA: semi-essential AA (Cys+Tyr), NEAA: non-essential AA (Asp+Ser+Glu+Pro+Gly+Ala+Arg)

Effect of the season

As well documented in previous studies milk composition varies considerably throughout the seasons (*e.g.* Jahreis *et al.* 1996, Auldist *et al.* 1998, Lindmark-Månsson *et al.* 2003, Lock & Garnsworthy 2003, Litwińczuk *et al.* 2011). In our study, during the summer concentration of CP, CAS, TP and WP was lower than in the winter ($P<0.05$). On the other hand samples taken in the summer contained higher proportion of NPN in comparison to the winter ($P<0.05$). Similar findings were also reported by Kriščiunaite *et al.* (2012), Litwińczuk *et al.* 2011, Heck *et al.* (2009) who noted the lowest content of milk protein in summer and the highest in autumn/winter or by Lindmark-Månsson *et al.* (2003); however their milk differed in profile of nitrogen fractions. The mean content of TP in Swedish milk was 3.37 g/100 g and ranged from 3.32 g/100 g found in May to 3.45 g/100 g measured in September while TP content determined in our study was lower, being 3.06 g/100 g in summer and 3.17 g/100 g in winter. On the other hand the concentration of CAS closely resembled the values of raw Swedish dairy milk as determined in the above mentioned study. The concentration of WP in our study ranged from 0.48 g/100 g found in summer to 0.51 g/100 g found in winter and was lower than that determined by Lindmark-Månsson *et al.* (2003). Similarly, the concentration of NPN being 0.20 g/100 g in summer and 0.18 g/100 g in winter was lower than given in Lindmark-Månsson *et al.* (2003). This discrepancy can be explained by a long-term decrease in the CAS content and a corresponding increase in the content of WP in Swedish milk between 1970 and 1996 that indicate a significant change in the proportion of proteins in milk (Lindmark-Månsson *et al.* 2003).

The concentration of essential, semi-essential, and non-essential amino acids was not affected by the season ($P>0.05$). The content of individual amino acids found in our study was slightly lower than that determined by Lindmark-Månsson *et al.* (2003) in Swedish dairy milk with a CP content of 3.67 g/100 g. The concentration of individual amino acids did not differ significantly except for Met whose content was lower during the summer (0.887 g/kg) in comparison to the winter (1.038 g/kg, $P<0.05$). Further, the concentration of Phe tended to be lower ($P=0.0827$) and the concentration of Ala tended to be higher ($P=0.0652$) in summer than in winter. This is in disagreement with Lindmark-Månsson *et al.* (2003) who determined seasonal variations in all amino acids except for Pro and Tyr. This discrepancy can be caused by differences in the profile of nitrogen fractions in milk as mentioned above. Furthermore, the concentration of free amino acids can also contribute to differences found in the concentrations of total amino acids (Lindmark-Månsson *et al.* 2003, Csapó *et al.* 1995). The total of amino acids in milk comprises amino acids incorporated into proteins as well as free amino acids that can be derived from different sources such as from normal metabolic processes, from substances specific to certain kinds of feedstuffs that pass in small quantities from the digestive tract to the milk or from processes connected with health status of cows because the content of free amino acids is highly correlated with udder inflammation (Csapó *et al.* 1995).

Effect of the feeding type

Milk yield in grazed herds was lower than in non-grazed herds being 5 197.50 and 7 203.75 kg, respectively ($P<0.05$). This is in agreement with other pasture-based studies (*e.g.* White *et* *al*. 2002, White *et al*. 2001, Kolver *et al*. 2000, Kolver & Muller 1998). The differences in milk production between grazed and non-grazed herds are likely related to differences in energy requirements for maintenance related to walking and grazing activity (Agnew *et al*. 2000), to differences in energy intake that is a primary limiting nutrient for high-yielding dairy cows on pasture (Bargo *et al*. 2002) and to differences in amounts, sources and ratio of rumen degradable protein and rumen undegraded protein for grazing cows (Eastridge 2006, Kolver & Muller 1998, DePeters & Cant 1992). As mentioned by Kolver & Muller (1998) the lower milk production from high producing dairy cows consuming only high quality pasture compared with cows consuming a nutritionally balanced total mixed ration (TMR) was due to a lower dry matter intake and energy intake.

The content of all studied protein fractions, which consists of CP, CAS, TP, WP and NPN in milk of grazed herds was lower than in non-grazed herds ($P<0.05$). Similar results were noted by Bargo *et al*. (2002) when comparing pasture, TMR and partial TMR based feeding systems while Jõudu *et al*. (2008) found a significant effect of pasture only on total CAS and some CAS fractions. On the other hand White *et al*. (2001) or Kriščiunaite *et al*. (2012) did not find any differences in the protein percentage between pasture and confinement cows or indoor and outdoor housing period, respectively. These contradictory results may have arisen from differences in the experimental design (Jõudu *et al*. 2008).

A total concentration of amino acids in milk of grazed herds being 26.690 g/kg was lower than in non-grazed herds – 31.252 g/kg ($P<0.05$). Similarly to the overall sum of amino acids, the concentration of essential, non-essential and semi-essential amino acids was lower in grazed than in non-grazed herds ($P<0.05$). Most of the amino acids showed significant differences, reflecting the above mentioned variation in the major milk proteins. The individual amino acid concentrations of the following amino acids were found to be lower in grazed than in non-grazed herds: Thr, Val, Ile, Leu, Phe, His, Lys, Tyr, Asp, Ser, Glu, Ala, Arg ($P<0.05$), further concentration of Cys also tended to be lower in grazed in comparison to non-grazed herds ($P=0.0818$).

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