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Amino acid composition of milk from Finnish Ayrshire cows and their crossbreeds with the Norwegian Red breed

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Abstract. The purpose of this work was to study the effect of crossing of Finnish Ayrshire cows with Norwegian Red breed under the conditions of the temperate climate of Ukraine on indicators of the qualitative composition of milk, the content of essential amino acids (EAA) and their biological value. The research was conducted at a commercial farm in the Poltava region (50°02′39″ n.l., 33°51′09″ e.l.) using Finnish Ayrshire cows and their crosses with the Norwegian Red breed. According to the indicators of fat, protein and lactose content in the group of crossbred firstborns, purebred counterparts prevailed by: 0.22; 0.09 and 0.07%, respectively, and the energy value of 1 kg of milk by 0.053 Mcal kg^{-1}. For milk protein of Finnish Ayrshires, the first limiting EAA (lower than the recommended content in the reference protein) was methionine + cystine (affects the rate of clot formation during cheese making), the content of which was 96.3%. In the protein of crossbred cows EAA in which amino acid score (AAS) was less than 100%, were not detected. Phenylalanine + tyrosine - 143.7% and leucine - 122.1%, which are aromatic amino acids and affect the taste properties of milk, were the most excessive. The protein of crossbreds was characterized by a slightly higher value of the total utilitarian coefficient, compared to purebred counterparts (by 12.77%). Also, the aminogram of the crossbred group was closer to 'ideal'.

Key words: dairy cows, breeds, crossbreeding, milk content, milk protein, amino acids.

INTRODUCTION

Today, one of the urgent problems in dairy farming is obtaining raw materials (milk) suitable for further processing with a high content of quality indicators (Claeys et al., 2014; Lutsenko et al., 2021; Matvieiev et al., 2023). The composition and yield of milk are determined by genetic factors but also depend on other factors: age, stage of lactation, type of feeding, season, climatic conditions, method and conditions of maintenance (Rafiq et al., 2016; McDermott et al., 2017; Ruban et al., 2020; Ruban et al., 2022). Along with the main indicators of milk quality, such as fat, protein, lactose and dry matter, its mineral, fatty acid composition, as well as pro- and antioxidant properties are equally important (Borshch et al., 2018; Górska-Warsewicz, et al., 2019;

Tham et al., 2019; Wang et al., 2019). Buitenhuis et al., (2023) investigated the possibility for involvement of milk amino acid composition in the breeding program of Danish cattle breeds. Knowledge of these indicators allows determining the suitability of dairy raw materials for processing into certain types of products (Sneddon et al., 2016; Stojanovska, et al., 2018; Amalfitano et al., 2020).

For quick resolve of productive and reproductive traits and improvement of the qualitative milk composition in the breeding of dairy cattle, interbreeding has been used more often (Hazel et al., 2017; Borshch et al., 2019). This is especially true of the world's most widespread Holstein breed (Berry, 2021). However, crossbreeding is also used with the use of less widespread (local) breeds depending on the natural and climatic zone and national and commercial motives (Cielava et al., 2017; Jonkus et al., 2020; Borshch et al., 2021; Borshch et al., 2022).

Ayrshire and Norwegian Red breeds of cows are not among the most widespread in terms of numbers and with an even distribution on all continents, however, they are often used in the breeding of local breeds, or in cross-breeding with representatives of the *Bos taurus* species and the *Bos indicus* subspecies to improve reproductive traits, resistance to non-contagious diseases, sensitivity to low temperatures and quality milk composition (Galukande et al., 2013). These breeds are quite often used in three-breed crossbreeding (VanRaden et al., 2020).

Researches carried out in Tanzania (Chenyambuga & Mseleko, 2009), Kenya (Thorpe et al., 1993) and Burundi (Hatungumukama et al., 2007) using local cattle with Ayrshire showed reduced productivity of first-generation crosses.

However, Lopez-Villalobos et al. (2000) in their researches conducted in New Zealand indicated that Holstein × Ayrshire crosses were lower in position to purebred Holsteins not only in productivity (obtained from one hectare of pasture), but also in fat and protein content. In the same work, the authors report that Jersey × Ayrshire crossbreds surpassed purebred Jerseys in terms of productivity, but were inferior in terms of fat and protein content. McAllister et al. (1994) in research conducted in Canada reported that Holstein × Ayrshire crossbreeds in some cases were not worse then purebred Holsteins in terms of productivity and had higher indicators of lifetime profitability.

Ezra et al. (2016) indicate that purebred Holsteins prevailed both in terms of milk yield and fat and protein content in the milk of Norwegian Red × Holstein crosses.

Groups of scientists from Europe (Freyer et al., 2008; Sørensen et al., 2008; Benak et al., 2020; McClearn et al., 2020), Asia (Saravanan et al., 2021), Africa (Galukande et al., 2013), North America (Heins & Hansen, 2012; Hazel et al., 2021), Oceania (Lopez-Villalobos et al., 2021) report about improvement of the technological characteristics of milk when using interbreed crossing with the use of Ayrshire and Norwegian Red breeds. Sørensen (1995) demonstrated the heterosis effect on fat, and protein yield in F_1 (Danish Red × Brown Swiss) at the level of 8.4 and 8.2% respectively compared to purebred Danish Red cows. The heterosis effect on fat content at 1.7% in crossbred Swedish Friesian × Swedish Red was established (Ericson et al., 1988). Vance et al., (2013) reported about significant effect of crossbreeding on fat and protein yield in dairy cows in grassland-based systems. It was noticed by Quénon & Magne (2021), that the protein and fat content in milk of crossbred cows (½ Holstein × ½ Montbéliarde, ½ Holstein × ½ Viking Red, ½ Holstein × ½ Simmental, and ½ Holstein × ½ Brown Swiss) were higher than in purebred Holstein cows. Crossbreeding of local German Black and White breed with Danish Jersey had a have an influence on the rise of protein and fat content

of milk compared to local purebred (Panicke & Freyer, 1992). However, there are no references in the literature about the effect of crossing Ayrshire and Norwegian Red breed on the parameters of fat, protein, lactose and essential amino acids.

The purpose of this work was to study the effect of crossing Finnish Ayrshires with Norwegian Red breed in the temperate climate of Ukraine on milk quality indicators, the content of essential amino acids and their biological value.

MATERIAL AND METHODS

The investigation was conducted in the central part of Ukraine, specifically within the Poltava region at geographic coordinates 50°02′39″ N latitude and 33°51′09″ E longitude. The subjects of the study comprised purebred Finnish Ayrshire cows and first-generation crossbreds of Finnish Ayrshire and Norwegian Red breeds. These animals were housed in a loose housing system within a modular structure featuring deep straw litter. Milking was carried out twice daily using a 'Parallel' 2×12 milking parlor. The study population encompassed two distinct groups: purebred cow (Finnish Ayrshire, $n = 16$) and crossbred cow (F_1 ½ Finnish Ayrshire × ½ Norwegian Red, $n = 20$), all of which were primiparous. Animals were in the third month of lactation for 74 ± 17 days and were not pregnant.

Cows fed total mixed ration. Distribution of feed took place twice a day (at 09.00 and 19.00 hours).

Milk samples were collected over a two-day period, encompassing both morning and evening milkings. The Milkotester device (Lactomat RapidS, Bulgaria) was employed to determine the fat, protein, and lactose content in the milk samples. The sampling procedure took place in June 2021. It should be noted that the average daily temperature during this period, which was +17.4 °C and fell within the thermoneutral range for dairy cows as defined by West (2003).

Optimal ratio of essential amino acids in the milk protein of the cows of the studied groups was determined by comparing their content with the aminogram of the standard form (methionine + cystine) of the 'ideal' chicken egg protein (WHO, 2007).

The amino acid compositions of milk proteins were analyzed and evaluated at the State Research and Development Control Institute of Veterinary Preparations and Feed Supplements in Lviv. The analysis was conducted using the capillary electrophoresis method with the 'Kapel 105/105M' system (Strus, 2015).

The amino acid score (AAS, %) of milk protein was calculated based on the percentage of each essential amino acid (EAA) in the milk protein relative to its content in the 'ideal' protein, which is typically represented by chicken egg, soy, or human milk protein (Schaafsma, 2000).

Biological value of milk protein was assessed using the Protein Digestibility Corrected Amino Acid Score (PDCAAS), a recommended method for evaluating protein quality by the Joint FAO/WHO Expert Council (WHO, 2007; FAO, 2013). The biological value was determined using the utility coefficient (U). The utility coefficient of each essential amino acid (EAA) in cow's milk was analyzed to estimate the protein's overall nutritional value:

$$a_1, \% = \frac{\mathrm{AA_{min}}}{\mathrm{AA_i\ \ EAAs\ of\ milk\ protein}} \quad (1)$$

where a_1 – utility coefficient of each EAA; AA_{min} – the minimum of chemical score of amino acids; AA_i EAA – the amino acid score of each EAA of milk protein.

Based on the obtained data, the coefficient of the total utility of EAA of milk proteins (U) was computed:

$$U = \frac{\sum \text{content}_i \text{ EAA} \cdot AA_i \text{ EAA} \cdot_{ai} \text{EAA}}{\sum \text{content EAA}, \frac{mg}{g} \text{ of milk protein}} \quad (2)$$

where $_i$EAA – each EAA (mg g^{-1} of protein); AA_i EAA – amino acid score of each amino acid; $_{ai}$ EAA – utility coefficient of each EAA.

The NEL of milk was estimated using an equation by the NRC (2001):

$$\text{NEL (Mcal kg}^{-1}) = 0.0929 \times \text{fat, \%} + 0.0547 \times \text{protein, \%} + 0.0395 \times \text{lactose, \%} \quad (3)$$

where NEL is the gross energy of one kg of milk.

The data are expressed as means ± standard error of the mean. To assess statistical significance, Student's *t*-test was employed, with significance levels indicated as $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. The statistical analyses were conducted using STATISTICA software (Version 11.0, 2012).

RESULTS AND DISCUSSION

The data in Table 1 indicate higher indicators of daily yield fat, protein and lactose content in the group of crossbred firstborns compared to purebred Finnish Ayrshires by: 0.061; 0.015 and 0.008%, respectively. In addition, the energy value of 1 kg of milk was also higher: by 0.053 Mcal kg^{-1}.

Table 1. Milk yield and composition of different breeds cows

Indicators	Finnish Ayrshire	Finnish Ayrshire × Norwegian Red
Milk yield, kg day^{-1}	21.38 ± 0.37	21.16 ± 0.26
Fat yield, kg day^{-1}	0.732 ± 0.034	0.793 ± 0.026*
Protein yield, kg day^{-1}	0.683 ± 0.007	0.698 ± 0.009*
Lactose yield, kg day^{-1}	0.975 ± 0.003	0.983 ± 0.003*
Energy value of 1 kg of milk, Mcal kg^{-1}	0.660 ± 0.011	0.713 ± 0.015*

*$P < 0.05$ as compared with Finnish Ayrshire group.

Puppel et al., (2017) report about the positive influence of the Norwegian Red breed of cows when crossed with Holsteins of Polish breeding on the indicators of the qualitative composition of milk. Ferris et al. (2014) in their research conducted in Ireland also reported about increased fat + protein content in Holstein-Friesian crosses with Norwegian Red compared to a group of purebred cows. Similar data were also obtained in the study of Benak et al., (2020) conducted in Croatia. The result of the present investigation was similar to Shortall et al., (2018), which showed that the milk yield of crossbreed cows (Norwegian Red × Holstein-Friesian) was slightly lower than the milk yield of purebred (Holstein-Friesian).

As per the recommendations of FAO/WHO, when assessing the biological value of milk proteins, it is customary to compare the amino acid composition of the proteins being studied with that of the 'ideal' protein. It is essential for the studied protein to not

only contain sufficient amounts of essential amino acids (EAA), but also have a ratio between individual essential amino acids that closely matches the ratio found in human body proteins. The amino acid score (AAS, %) of milk protein, calculated by comparing the percentage ratio of each EAA in milk protein to its content in the 'ideal' protein, is presented in Table 2. For Finnish Ayrshire milk protein, the first limiting amino acid was methionine + cystine, which significantly affects the rate of clot formation during cheese making, with a content of 96.3% compared to the adequacy scale in the 'ideal' protein. In the protein of crossbred cows, no amino acids were found to have an AAS less than 100%, indicating that the content of each EAA met the human requirements in the reference protein. Phenylalanine + tyrosine (143.7%) and leucine (122.1%), which are aromatic amino acids and influence the taste properties of milk, were found to be in excess.

Table 2. Amino Acid Score (AAS) of Milk Protein in Cows of Different Genotypes Compared to the 'Ideal' Protein

EAA	EAA content in the 'ideal' protein, mg per g‡	Finnish Ayrshire		Finnish Ayrshire × Norwegian Red	
		EAA, mg g^{-1}	AAS, %	EAA, mg g^{-1}	AAS, %
Lysine	55	60.3 ± 0.48	109.6	62.8 ± 0.17***	114.2
Methionine + cystine	35	33.7 ± 0.35	96.3	36.0 ± 0.62***	108.8
Threonine	40	43.4 ± 0.12	108.5	43.8 ± 0.37	109.5
Valine	50	54.3 ± 0.23	108.6	56.1 ± 0.40**	112.2
Leucine	70	83.8 ± 0.57	119.7	85.5 ± 0.39*	122.1
Isoleucine	40	44.2 ± 0.34	110.5	46.4 ± 0.31***	116.0
Phenylalanine + tyrosine	60	82.1 ± 0.78	136.8	86.2 ± 0.93***	143.7

‡ – FAO/WHO Scale of EAA Adequacy with Respect to Human Needs. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ as compared with Finnish Ayrshire group.

Previous research has investigated the impact of crossbreeding on the amino acid composition of milk in first-generation hybrids obtained by crossing Bos indicus subspecies with Bovinae subfamily representatives. Mapekula et al. (2011) found that crossbred Nguni cows, when crossed with local South African cows, exhibited lower levels of methionine, threonine, tyrosine, glycine, and proline in their milk protein composition compared to purebred Nguni cows. On the other hand, Sun et al. (2014) and Ren et al. (2015) conducted studies in the Guangxi Autonomous Region of China and observed that crossbred river buffalo × swamp buffalo hybrids displayed higher levels of essential amino acids (excluding cystine) in their milk protein composition compared to purebred river buffalo. Olsen et al. (2021) reported that the milk which was conducted from Norwegian red cows was characterized by a higher content of fatty acids and good quality for producing Norwegian Gouda-type cheese. The similar results were obtained by Csapó et al. (2012). The lysine content in the milk of F1 cows was higher than in purebred, instead significant heterosis effect on the content of other essential amino acids wasn't indicted (McDermott et al., 2017). It was found that the biological value of cow's milk calculated by the Morup-Olesen index (Morup & Olesen, 1976) in the Ayrshire breed was lower than in the Norwegian red by 3 points (Csapó et al., 2011).

One important parameter that characterizes the nutritional quality of proteins is their digestibility or utilization. The utilitarian coefficient of the amino acid composition measures the balance of essential amino acids (EAA) relative to the physiological

reference value. A higher utilitarian coefficient indicates a better balance of amino acids within the protein and suggests a more efficient utilization by the body. Table 3 presents the results of calculating the utility coefficient for each EAA in the milk protein of cows from different breeds.

Based on the data presented in the table, it is evident that both experimental groups of cows exhibit a significantly high level of balanced amino acid composition, as indicated by the value of the total utilitarian coefficient. This implies that the content of essential amino acids (EAA) in the protein, which are crucial for fulfilling the constructive requirements of the human body, is considerably substantial. Notably, the crossbred Finnish Ayrshires with the Norwegian Red-breed display a relatively larger total utilitarian coefficient of 108.80%, which is 12.77% higher than that of purebred Finnish Ayrshires.

Table 3. Utility Coefficients of Milk Protein in Cows of Various Genotypes

EAA	Finnish Ayrshire	Finnish Ayrshire Norwegian Red
Lysine	0.88	0.95
Methionine + cystine	1.00	1.00
Threonine	0.89	0.99
Valine	0.88	0.97
Leucine	0.80	0.89
Isoleucine	0.87	0.94
Phenylalanine + tyrosine	0.70	0.76
Overall Utility coefficient	96.03	108.80

To find out the optimal ratios of EAA in the milk protein of cows of the studied breeds, their content was compared with the aminogram of the standard form (methionine + cystine) of the 'ideal' chicken egg protein (WHO, 2007). Comparison with the 'ideal protein' aminogram, milk of the studied breeds did not contain amino acids, the ratios of which less than optimal (Table 4).

Table 4. Amino Acid Formula of Cows of Different Breeds According to Methionine+Cystine and Human Needs

Breeds	Optimal formula for chicken egg protein						
	Met+Cys	Lys	Thr	Val	Leu	Ile	Phe+Tyr
	1.0	1.57	1.14	1.43	2.00	1.14	1.71
Finnish Ayrshire	1.0	1.79	1.29	1.61	2.48	1.31	2.43
Finnish Ayrshire × Norwegian Red	1.0	1.74	1.21	1.56	2.37	1.29	2.39

The closest to 'ideal' one the aminogram of milk protein of Finnish Ayrshire crossbred cows with Norwegian Red was, and the maximum differences were observed in the aminogram of milk protein of purebred Finnish Ayrshires. In this research was obtained data as in the study by Borshch et al., (2019), where amino acid composition in cows milk of crossbreds of local breeds with Brown Swiss and Montbeliarde breeds were investigated.

CONCLUSIONS

The use of an intercross breeding between Finnish Ayrshires and Norwegian Red breed had a positive effect on the content of fat, protein and lactose in milk (+0.22, 0.09 and 0.07%, respectively). The milk protein of crossbred cows had a higher biological value compared to purebred animals. For the milk protein of Finnish Ayrshire cows, the

first limiting amino acid was methionine + cystine, the content of which was 96.3%. The milk protein of crossbred cows did not contain amino acids, amino acid score of which was less than 100%. Crossbreds have a higher value of the total utilitarian coefficient: by 12.77% compared to purebred Finnish Ayrshires. The milk protein of cows of the studied breeds does not contain amino acids, the ratio of which is less than the optimal one, compared to the amino acid formula of compliance with human needs according to FAO/WHO. The milk of crossbreeds of Finnish Ayrshires and Norwegian Red cows was the best in terms of quality and protein composition, which gives reasons to consider it the most suitable for the production of cheeses and sour milk products compared to purebred counterparts.

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