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THE INFLUENCE OF GENETIC VARIANTS OF κ-CASEIN ON MILK COMPONENTS

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ABSTRACT

Milk production of 22 cows of Slovak Pied breed with Holstein-Friesian was analyzed according to the genetic variants of the polymorphic proteins determined by starch gel electrophoresis. The effect of genetic variants of the proteins was analyzed by selected properties of milk (milk yield, proteins, fats and lactose). Differences between the productive characters in testing groups were evaluated according to statistic method of t-test. Evaluation was carried out during throughout lactation. Based on the analyses we have obtained results frequency of genotypes: κ-CN AA in 9 cows (41%), AB in 12 cows (54.5%) and BB in one cow, which is 4.5%. The average daily milk production of κ-CN AA was 13.5 l/day and in κ-CN AB 14.2 l/day. Contents of protein of genetic variation κ-CN AA was 3.1% in milk genotype κ-CN AB was found not significant lower protein proportion 3.0%. Based on the analyses, we can assume that cow's nutrition higher influence the increase in the proportion of protein than polymorphism of κ-CN. In our research was found out the average fat content 4.0% in genetic variation of κ-CN AA and not significant lower in genetic variation κ-CN AB 3.8%. The average lactose content in the cow's milk with κ-CN AA genotype was 4.9% and κ-CN AB was 5.0%. The difference between fat content wasn't statistically significant.

Keywords: κ -casein, proteins, polymorphism, milk yield, milk composition

INTRODUCTION

Caseins participated to phosphorproteins that account for nearly 80% of bovine milk proteins and whey proteins the other 20% **(Lucey *et al.*, 2003).** Casein is a general term for class of proteins and casein is further divided into specific proteins such as αs_1-casein, αs_2-casein, β-casein and κ-casein (CSN1S1, CSN2, CSN1S2 and CSN3, respectively) **(Farrel *et al.*, 2004).** Caseins form very large spherical complexes in milk called casein micelles. Compared to fat globules the casein micelle is about ten to one hundred times smaller.
α-casein actually comprises two fractions: αs_1-casein and αs_2-casein. However, these two fractions are difficult to separate from each other. The four fractions αs_1-, β -, κ -, and αs_2-casein occurs in the weight ratio 3:3:1:0:8. All four fractions are phosphoproteins containing phospate groups and as a result are "calcium-sensitive" and may be coagulated by addition of calcium **(Agüera *et al.*, 2004).**
κ-casein contains only one phosphate group and is not calcium sensitive. The αs- and β -casein fractions are very hydrophobic. However, κ-casein is a glycoprotein containing an acidic (charged) carbohydrate section, and so it is much more hydrophilic. In milk, the casein fractions associate with each other and with colloidal calcium phosphate to form stable spherical structures known as casein micelles **(Prinzenberg *et al.*, 2005).** The more hydrophobic –αs and - β-casein fractions exist mainly on the micelle surface. It is the κ-casein that gives the micelles their stability in milk under normal handling conditions. This is due to the negative charge and hydration of the κ-casein, coupled with the fact that the charged and hydration of the κ -casein, coupled with the fact that the charged hydrophilic carbohydrate section of the molecule tends to protrude from the micelle surface in hair-like structure, which confer steric (or spatial) stability on the micelles. The casein micelles are coagulated by addition of acid at a pH of 4.6-5.2. As the micelles approach their isoelectric point, the charge and extent of hydration is reduced, and the κ -casein hair-like structure flatten, reducing steric hindrance **(De Kruif *et al.*, 2003).** Hence, the micelles are no longer stable and so they aggregate. This is the basis for the formation of cottage cheese, which is an acid cheese containing casein curds. Acids also cause some calcium to be removed from the micelles, and so cottage cheese is relatively low in calcium compared with some other dairy products. Casein contains both hydrophobic and hydrophilic sections, in addition, they contain a high proportion of the amino acid proline, and so they are flexible proteins containing little regular, ordered secondary structure. As a result, they readily adsorb at an oil-water interface, forming a stable film which prevents coalescence of emulsion droplets, thus they make excellent emulsifiers **(Chiatti *et al.*, 2007).** *κ*-Casein, which represents approximately 15% of total CN, is the most interesting and important CN component in that it is centrally involved in the formation and stabilization of CN micelles, the coagulation of milk by rennet, and many other technologically important functions of the milk protein system. The functional duality of *κ*-casein, which is to interact hydrophobically with other CN and at the same time provide a hydrophilic and negatively charged surface on the micelle to stabilize the colloidal suspension, is strikingly reflected by its amphipathic primary structure **(Swaisgood, 2003).** Genetic variability of milk proteins (κ-CN) is interesting in terms of research, breeding cattle and processing of milk for the positive influence of some alleles, particularly B allele of κ-CN, milk composition and technological properties **(Azevedo *et al.*, 2008). Mácha (1991)** adds the difference between the A and B variants of κ-CN consist of amino acid substitutions that cause the κ -CN B variant is more hydrophobic and milk with this variant can be obtained more cheese, time of renneting is shorter and cheese has better organoleptic properties. Technologically κ-CN B variant can be considered advantageous because dairy cow milk has a higher content of protein in cheese and processed milk provides higher yield about 5-10%. **Sitkowska *et al.* (2008)** confirm that κ-CN BB is more desirable than κ-CN AA because it is associated with a higher content of casein in milk. Also, higher cheese yield, shorter coagulation time and curd firmness higher. B allele of κ-CN causes the formation of small micelles, which results in lower losses of fat and protein in the whey, also, been observed positive relationship between B allele of κ-CN and fertility cattle. **Heck *et al.* (2009)** report that κ-CN B variant has a higher content of total protein on a 0.15 to 3% than κ-CN A variant. Some works confirm the beneficial effect of B allele of κ-CN on protein production only in the homozygous state. It was found the lowest protein content of genotype κ-CN AB. Polymorphism of milk proteins does not only affect the protein content of milk but also for its technological properties and cheese. The quality and yield of cheese given by the volume and type of casein in milk. Variants of κ-CN have significant effect influenced on the quality of the cheese. Milk containing the B variant of κ-CN is characterized by a greater yield of cheese curd better quality,

less fat loss in whey, milk protein coagulation faster and consumes less rennet. In the milk containing κ-CN BB is coagulation time shorter by 24% than in milk containing κ-CN AA, the consistency of the curd is firmer by 37% and the yield of the product is increased by 5-10% **(Wedholm *et al.*, 2006).**
The aim of this work was to determine the frequency of κ-casein genotypes and effects of κ-casein on the chemical composition of the milk.

MATERIAL AND METHODS

In this work were analyzed 22 samples of cow's milk crosses of Slovak Pied and Holstein-Friesian breeds. For the analysis milk samples were collected monthly throughout lactation. Milk (500 ml) were sampled, cooled to 5-10 °C in the refrigerator and were analyzed next day (14-16 hour after sampling).
In the laboratory were determined:

- Fat content (g/100ml)
- The lactose content (g/100 g)
- Protein content (g/100 g).

Fat, protein and lactose were analyzed quickly by analyzer MILKOSCAN 104 A/B. The essence of the method is the determination of milk constituents with infrared analyzer, which measures the amount of light absorbed in certain wavelengths molecules of fat, protein and lactose.

1. For the determination of fat content was measured amount of absorbed light by carboxy ester bonds of glycerides, at a wavelength of about $5.73x10^{-6}$ meters. Fat content was analyzed in g/100ml and calculated to g/100g.
2. For the determination of protein content was measured amount of light absorbed by secondary amide groups of peptide bonds, at a wavelength of $6.46x10^{-6}$ meters.
3. For the determination of lactose, at a wavelength of about $9.61x10^{-6}$ meters.

Preparation of samples

Samples were heated in a water bath at 35-40 °C, thoroughly mixed to make it homogeneous dispersed fat, but foaming of milk is undesirable. Sample was mixed with a stirrer of device once again for 5-10 seconds. The required amount for analysis (6 mL) was taken up by pump. Measured results were automatically displayed on the display and were written to the printer device.

Determination of casein fractions

Individual protein solutions were prepared separately by dissolving 125 mg of purified $\alpha s_1$and αs_2-casein, 100 mg of β-casein or 25 mg of κ-casein in 5 ml of a solution containing 8 M urea, 165 mM Tris, Trips 44 mM sodium nitrate and 0.3% β-mercaptoethanol. A mixed standard solution was prepared by mixing 1 ml of each individual protein solution and adding 2 ml of urea solution, and then adding urea solution to a final volume of 5 ml. Further dilution was carried out to produce a final casein concentration of 2 mg mL−1. Twenty microlitre was then directly injected into the HPLC system. The casein fractions were quantified using RP-HPLC system. The milk samples were prepared for analysis by diluting 0.5 ml skimmed milk with 1.5 mL of urea solution. The diluted samples were filtered with a 0.45 μm PVDF filter and analysed in a Dionex system consisting of P680 HPLC pump, ASI – 100 automated sample injector, thermostatted column compartment TCC100, PDA – 100 photodiode Array Detector with a Jupiter C4 column (250x4.6 mm, 300 Å –sized pores, 5 μm sized particles; Phenomenex, Macclesfield, Cheshire, UK). The elution was performed at 25 °C with linear gradient B (0.1% TFA in acetonitrile) in A (0.1% TFA in water) from 30–50% in 45 min at a flow rate of 1.0 ml min^{-1}. Peaks were detected at the wavelength of 220 nm.

RESULTS AND DISCUSSION

The work presents results of analyzes of milk 22 cows, crosses of the Slovak Pied breed with Holstein-Friesian. Based on the analyses we have obtained results frequency of genotypes: κ-CN AA cows 9 (41%), AB in 12 cows (54.5%) and BB in one cow, which is 4.5%**. Mederano *et al.* (1991)** presents the similar representation of genotype frequencies. The highest incidence was observed in κ-CN AB (54.7%), lower in κ-CN AA (23.4%) and the lowest in κ-CN BB (21.9%).

Table 1 The average milk production of genetic variants of κ-CN AA and κ-CN AB

Parameters		Milk yield (l)	Proteins (%)	Fats (%)	Lactose (%)
κ-CN AA	n	9	9	9	9
	mean	13.5	3.1	4.0	4.9
	S.D.	2.17	0.27	0.49	0.24
	CV%	16.1	9.0	12.4	5.0
K-CN AB	n	12	12	12	12
	mean	14.2	3.0	3.8	5.0
	S.D.	2.99	0.23	0.80	0.28
	CV%	21.4	7.7	21.6	5.6

Legend: n – number, S.D. – standard deviation, CV % – coeficient of variation

The evaluation of the average daily milk yield (Table 1) shows that cow's milk production of κ-CN AA was 13.5 l/day and in κ-CN AB 14.2 l/day. Figure 1 shows the average daily milk yield of genotypes of κ-CN during lactation.
Similar to our findings are the result of **Michalcová *et al.* (1997)**, who report higher milk production in genotype κ-CN AB than κ-CN AA however, the difference is not statistically significant. Also **Aleandri *et al.* (1992)** found out insignificant impact of κ-CN AA and κ-CN AB for milk production in agreement with our results. The nutritional and technological point of view, proteins are considered one of the most important and valuable components of cow's milk. Protein content (Table 1) of genetic variation κ-CN AA was 3.1%, in milk with genotype κ-CN AB was lower (3.0%) but difference wasn't significant. Figure 2 shows the average protein content of genetic variants of κ-CN in each month of lactation. Between κ-CN genotypes weren't found statistically significant differences. **Hynková, Havlíček (1996)** found out the highest percentage of protein in dairy cows with genotype κ-CN AA (3.3%) and lowest in κ-CN AB (3.1%) in milk of Holstein breed. Several authors **(Bovenhuis *et al.*, 1992; Ng-Kwai-Hang, 1995)** state that variant κ-CN B associated with increased protein content in milk. This view is confirmed by the results **Aleandri (1990)** who argue that Holstein-Friesian protein content was positively influenced by the genotype κ-CN BB. The average protein content in each month of lactation (Figure 2) shows the balance between κ-CN genotypes AA and AB. We found a gradual increase in the proportion of protein until the eighth month of lactation. Analysis of the average protein content (Figure 2) demonstrated the decreasing of protein content in the genetic variation of κ-CN AB in the month of May (4^{th} month of lactation**). Foltys (1997)** also indicated a significant decrease of protein in May (irrespective of the effect of genotype κ-CN). Since June (5^{th} month of lactation) reported a gradual increase in the proportion of protein in both genotypes κ-CN. The highest protein content of 3.2% was found out in September. Based on the analysis, we can assume that nutrition has greater effect for the increase in the proportion of protein than polymorphism of κ-CN. Minimum fat content is 3.3 % according to STN 57 0529. In our experiment, we found out the average fat content 4.0% in genetic variation of κ-CN AA and in genetic variation κ-CN AB 3.8% wasn't significant lower. Figure 3 shows the average fat content of genetic variants of κ-CN AA and AB in each month of lactation. Between κ-CN genotypes weren't found statistically significant differences. **Žitný *et al.* (1996)** found out in cows with genotype κ-CN AA higher production of milk fat compared with the κ-CN genotype AB and BB, even though the difference wasn't statistically significant. The average lactose content (Table 1) in the milk of cows with κ-CN AA genotype was 4.9% and κ-CN AB to 5.0%. The difference wasn't statistically significant. Figure 4 shows the average lactose content of κ-CN genotypes in each month of lactation. Between κ-CN genotypes were significant differences, however, in both genotypes, we found out a decrease of lactose in the second half of lactation, while in the last month was rapid increase in its content in both genotypes.

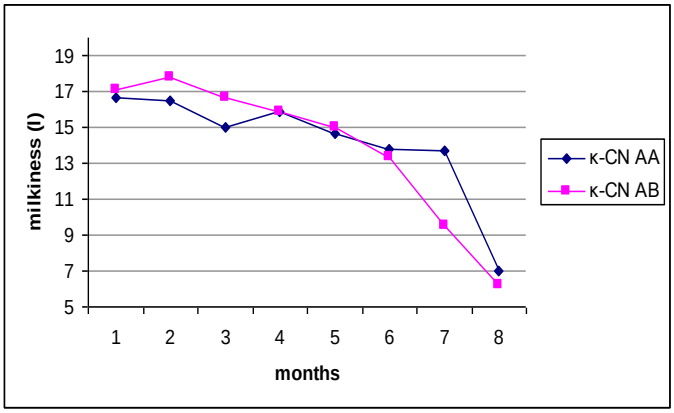

Figure 1 Average milk yield of genetic variant κ-CN in the lactation

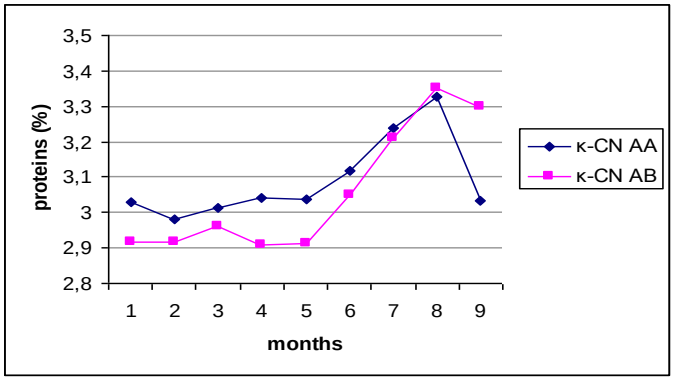

Figure 2 Average proteins content of milk genetic variant κ-CN in the lactation

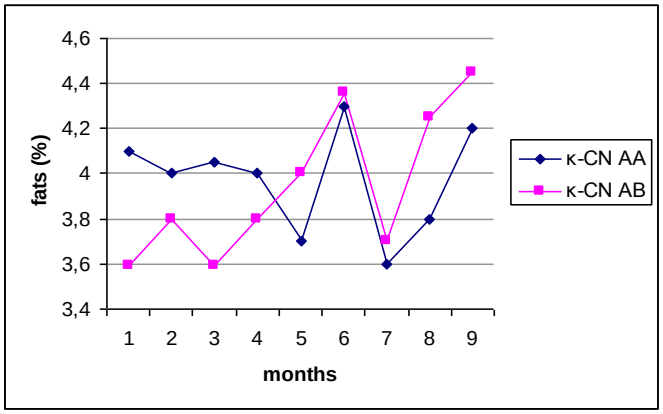

Figure 3 Average fats content of milk genetic variant κ-CN in the the lactation

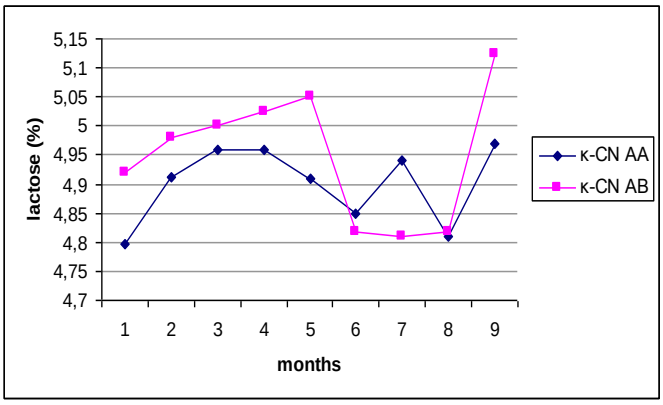

Figure 4 Average lactose content of milk genetic variant κ-CN in lactation

CONCLUSION

Based on our results and results of other authors, we can conclude that from the aspect of improving the technological quality of milk would be appropriate to increase the frequency of B allele of κ-CN. Our results show that dairy cows with κ-CN genotype AA are lower in milk production (13.5 l/day) opposite cows of genotype κ-CN AB (14.2 l/day). Proteins are one of the most important and valuable components of cow's milk. We found the average protein content in genetic variation of κ-CN AA 3.1% and genetic variation of κ-CN AB was 3.0%. The protein content during the lactation shows the similar balance in AA and AB κ-CN genotypes. We found out a gradual increase in the proportion of protein until the eight month of lactation. Based on the analysis and other authors we can assume that nutrition has a greater impact for the increase in the protein content than polymorphism of κ-CN. In our research, we found out the average fat content in genetic variation of κ-CN AA 4.0% and lower in genetic variation κ-CN AB (3.8%). The average lactose content in cow's milk with κ-CN AA genotype was 4.9% and κ-CN AB was 5.0%.

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