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COWS' MILK COMPOSITION IN RELATION TO AGE, LACTATION STAGE AND GENETICALLY MODIFIED FEED

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Abstract. The aim of the presented study was to analyze changes in milk composition depending on presence of genetically modified (GM) components in feed and according to cows' age and lactation phase. The research was carried out with Polish Holstein-Friesian black and white cows (n = 50) housed in tie stall system at one farm. The genetically modified feed was a supplementary feed mixture. Data reports from milking trials with milk chemical composition, number of somatic cells and urea content were collected. The collected data concerned groups: primiparous (P) cows and multiparous (M) cows during 2nd and 3rd lactation. In each age group, two subgroups were distinguished: cows from 1st to 4th month of lactation (I) and cows from 5th to 8th month of lactation (II). The above subgroups were divided according to feed with GM components (G) and feed with non-GM components (N). It was found that use of feed with the addition of GM plants contributes to lowering the level of casein and an increase of lactose in milk. Presence of GM components in feed did not affect the level of fat, total protein, fat to protein content, urea levels, somatic cell counts in milk and cow yield. It can be concluded that the withdrawal of feed additives based on GM components does not adversely affect the technological quality of milk.

Key words: genetically modified feed, milk composition, cow's age, lactation stage, dairy cattle.

INTRODUCTION

In recent years, the area of cultivated genetically modified (GM) crops has increased. In year 2014, the area included 181.5 million hectares of transgenic plants and in year 2017 this area enlarged to 189.8 million hectares (Clive 2015; ISAAA 2017). The interest in GM crops is related to a higher demand for fodder of plant origin (Sung et al. 2006). The use of molecular techniques and plant genome editing allows for the filling of the global feed deficit. However, there is still limited research determining the safety of genetically modified forage crops that are used in animal nutrition and other animals (Giraldo et al. 2019).

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Moreover, some research indicate that the consumption of products derived from animals that have consumed GM feed may have implications on human health (Shen et al. 2022). Therefore, the European Union has imposed restrictions on the cultivation of GM plants. It aims to prohibit production, commercialization, and use of GM fodder in animal feeding, as well as GM organisms intended for feed use (EU 2001, 2015). In the event that GM feed is used, a risk assessment must be carried out to ensure optimal animal production while avoiding any negative health effects on the animals (Mullins et al. 2023). Cow's milk, due to its nutritional value, is an important component of human diet. It is characterized by high bioavailability and biological activity with pro-health properties for humans and is treated as functional food (Spek et al. 2013; Výrostková et al. 2021). The composition of cow's milk is influenced by many factors, including animal feeding, age and lactation stage (Rodríguez-Bermúdez et al. 2023). The first contact with GM feed occurs in the digestive tract, and in addition, in cows, the processes occurring in the rumen affect the health of the animals and the quality of animal-derived products. According to Sung et al. (2006), transgenic maize does not affect the fermentation profile in cattle's rumen. Therefore, it is important to conduct research describing the impact of GM feed on the quality of animal-derived products. The aim of the presented study was to analyze changes in cows' milk composition depending on presence of GM components in feed and according to cows' age and lactation phase.

MATERIALS AND METHODS

The research was carried out from January to December in the herd of Polish Holstein-Friesian black and white cattle ($n = 50$) included in milk performance assessment conducted by the Polish Federation of Cattle Breeders and Milk Producers (milk recording). Animals were housed in tie stalls system at one farm. Cows were fed with use of TMR system as follows: maize silage (56.85%), sugar beet pulp (10.20%), grass silage (21.99%), grain middlings (8.16%) and supplementary feed mixture (2.8%) which included maize, canola meal, triticale, corn distillers grain, soybean meal (with GM or non-GM components), malt culms, palm oil, calcium carbonate, magnesium oxide, sodium chloride. Daily feed dose per cow was 7 kg. Animals were fed with GM supplementary feed mixture from January till May. Since June animals stopped to receive GM components in feed. The change of complementary feed was required by milk-collecting dairy factory. The food ration was based on INRA standards (IZ-INRA 2009).

The analysis of milk parameters was based on monthly data reports (10 reports) from milk recording performed with the AT4 method. Data set of two reports, from June and July, were excluded due to the normalization of physiological processes in the animals associated with the change of feed. Moreover, the data of 19 cows culled from the herd during the experiment were not taken into consideration. In order to analyze data from monthly reports, the results of the remaining animals were classified into the following groups: primiparous (P) cows and multiparous (M) cows during 2nd and 3rd lactation. In each age group, two subgroups were distinguished: cows from 1st to 4th month of lactation (I) and cows from 5th to 8th month of lactation (II). The subgroups were divided according to feeding: with GM components (G) and non-GM components (N). The analysis included: daily milk yield per cow (Kg), milk composition (% fat, % protein, % lactose, % casein, % dry matter), somatic cells count (thous. cells/mL), urea (mg/L) and the ratio of the percentage of fat to the percentage of protein in milk.

Statistical analysis was performed with Statistica 13.1. software (StatSoft Polska, Kraków, Poland). To determine the impact of cows' age, lactation stage and feeding on analyzed traits multivariate analysis of variance (ANOVA) and Tukey's post hoc test were used. Results are expressed as the mean \pm SD. Differences were considered significant at $P < 0.05$ or highly significant at $P < 0.01$.

RESULTS AND DISCUSSION

Higher level of fat was found in milk from primiparous cow's in 2nd stage of lactation, in non-GM group (PIIN) compared to milk from primiparous cow's fed with GM components, regardless of lactation phase (PIG, PIIG; $P < 0.05$). Moreover, cows from PIIN group had lower lactose level than animals from PIG and PIIG groups ($P < 0.01$). The composition of milk is affected by age of cows and lactation stage (Kęsek et al. 2017; Zielak-Steciwko et al. 2017). Obtained results from the presented study show that there might be a relation between the use of GM feed in cattle nutrition and level of lactose in cows' milk. Highly significant differences were observed in multiparous cows, in 1st stage of lactation, animals from non-GM group had higher casein level than animals from GM group ($P < 0.01$). An opposite relation was observed concerning dry matter in multiparous cows, in 2nd stage of lactation, when cows from GM group had higher dry matter content than cows from non-GM group ($P < 0.05$). Interestingly, during the 1st phase of lactation multiparous cows fed with GM components had higher milk production compare to cows fed with non-GM ($P < 0.05$). More detailed results are presented in Table 1 and Table 2.

Table 1. Milk composition (mean \pm SD) of primiparous (P) and multiparous (M) Polish Holstein-Frisian cows fed with genetically modified components (G) and fed with non genetically modified components (N) depending on cow's age and lactation phase

Milk composition	Group								P-value			
	P				M				A	S	F	
	I		II		I		II					
	G	N	G	N	G	N	G	N				
Fat [%]	\bar{x}	4.14 ^a	3.63	3.87 ^c	4.82 ^{Aab}	3.97 ^B	4.22	4.77 ^{Ab}	4.18	0.21	<0.01	0.94
	SD	0.86	0.30	0.97	0.78	0.70	0.66	0.53	0.61			
Total protein [%]	\bar{x}	3.32 ^{BCac}	2.97 ^{BCa}	3.68 ^b	3.63 ^b	3.25 ^{Bc}	3.52 ^{ac}	3.62 ^{Cbc}	3.76 ^{AC}	<0.05	<0.00	0.99
	SD	0.28	0.15	0.33	0.26	0.29	0.47	0.25	0.48			
Casein [%]	\bar{x}	2.59 ^{ACa}	2.62	2.78	3.09 ^{Bb}	2.51 ^c	2.79 ^{ABb}	3.01 ^B	2.88 ^B	0.63	<0.00	<0.02
	SD	0.16	0.36	0.20	0.20	0.24	0.39	0.30	0.32			
Lactose [%]	\bar{x}	4.93 ^B	4.90	4.98 ^B	4.72 ^A	4.82	4.84	4.76 ^A	4.75 ^A	<0.00	<0.02	<0.01
	SD	0.18	0.04	0.16	0.23	0.11	0.12	0.15	0.13			
Dry matter [%]	\bar{x}	12.99 ^{BCab}	12.44 ^b	12.98	14.00 ^{Cc}	12.72 ^B	13.18 ^{bc}	13.96 ^{Aac}	13.18 ^{bc}	0.35	<0.00	0.83
	SD	0.86	0.64	1.17	0.89	0.92	0.87	0.70	0.89			
Fat : protein	\bar{x}	1.21	1.11	1.08	1.24	1.23	1.22	1.25	1.16	0.14	0.76	0.74
	SD	0.20	0.06	0.22	0.17	0.20	0.22	0.13	0.11			

Lowercase letters indicate statistically significant differences at the level of $P < 0.05$ in rows. Uppercase letters indicate statistically significant differences at the level of $P < 0.01$ in rows.

I – cows from 1st to 4th month of lactation; II – cows from 5th to 8th month of lactation; A – age; S – stage of lactation; F – feed.

Table 2. Mean \pm SD of performance, urea level and Somatic Cell Count (SCC) in milk from primiparous (P) and multiparous (M) Polish Holstein-Frisian cows fed with genetically modified components (G) and fed with non genetically modified components (N) depending on cow's age and lactation phase

Parameters		Group								P-value		
		P				W				A	S	F
		I		II		I		II				
		G	N	G	N	G	N	G	N			
Performance [kg]	\bar{x}	27.22 ^{BCa}	30.23	25.08 ^{BC}	22.26 ^{BC}	34.16 ^A	28.14 ^B	20.30 ^{Cb}	25.00 ^{BC}	0.57	<0.00	0.82
	SD	4.41	5.09	5.79	5.25	8.01	8.39	5.79	4.99			
Urea [mg/L]	\bar{x}	232.13	238.25	253.60	238.56	237.58	208.62	232.77	214.80	0.11	0.58	0.187
	SD	39.83	81.51	62.33	42.30	56.29	61.84	48.66	62.70			
SCC [thous. cells/mL]	\bar{x}	231.57	51.75	414.20	748.81 ^A	88.00 ^B	437.85	441.00	388.73	0.84	<0.01	0.33
	SD	509.94	31.60	843.66	693.53	104.94	901.05	695.67	466.61			

Lowercase letters indicate statistically significant differences at the level of $P < 0.05$ in rows. Uppercase letters indicate statistically significant differences at the level of $P < 0.01$ in rows.

I – cows from 1st to 4th month of lactation; II – cows from 5th to 8th month of lactation; A – age; S – stage of lactation; F – feed.

The chemical composition of milk determines the nutritional value and technological properties of milk and dairy products. The quality of cow's milk is conditioned by many genetic and environmental factors. Milk proteins represent a high nutritional and functional value of milk. Mainly, casein content have a significant impact on cheese yield (Gellrich et al. 2014; Vigolo et al. 2023). In the presented study, a tendency for reduced casein level with GM components was observed but this tendency was not significant. High quality technological cow's milk is characterised by casein level of 2.46 to 2.80% (Zicarelli 2004; Maxhuni et al. 2015). In present research, the level of casein in cow's milk of all groups was within or above this range. The obtained results may indicate that the milk from all cows, regardless of their diet, exhibited good technological quality, which may be associated with high cheesemaking efficiency (Vigolo et al. 2023). However, the use of GM feed resulted in a decrease in casein level, which is not a desirable characteristic but it can be stated that despite the observed minor changes in the composition of milk, the use of GM feed does not deteriorate the technological quality of the raw material. The transgenic maize and soybean meal used in cow's feeding does not affect the basic composition of milk (Brouk et al. 2011; Furgał-Dierżuk et al. 2015). Feeds based on GM plants do not disturb the activity of the rumen microbiome, which in turn does not affect the quality of milk (Wiedemann et al. 2007). These research suggest the lack of effect of GM feed on the milk basic composition. In present study, no relation was noted, the use of feed containing GM components did not affect in general the milk yield and the level of fat, total protein, fat to protein ratio, urea level and somatic cell count. This is consistent with results from comprehensive research project conducted by the National Research Institute of Animal Production, Balice, Kraków and National Veterinary Research Institute, Puławy in Poland (Impact of GMO feed on animal productivity and health, transfer transgenic DNA in the

digestive tract and its retention in tissues and foodstuffs of animal origin, 2012), concerning the influence of GM feed on livestock productivity and health.

CONCLUSIONS

Based on the obtained results, it can be stated that milk composition and cow's performance are significantly affected by lactation stage and cow's age. However, the use of complementary feed stuff with genetically modified components does not affect in general milk composition and milk performance.

REFERENCES

- Brouk M.J., Cvetkovic B., Rice D.W., Smith B.L., Hinds M.A., Owens F.N., Liams C., Sauber T.E.** 2011. Performance of lactating dairy cows fed corn as whole plant silage and grain produced from genetically modified corn containing event DAS-59122-7 compared to a nontransgenic, near-isogenic control. *J. Dairy Sci.* 94(4), 1961–1966. DOI: 10.3168/jds.2010-3477.
- Clive J.** 2015. Global status of commercialized biotech/GM crops: 2014. ISAAA Brief No. 49. Ithaca, NY: ISAAA.
- EU.** 2001. Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC (OJ L 106, 17.4.2001, p. 1).
- EU.** 2015. Directive (EU) 2015/412 of the European Parliament and of the Council of 11 March 2015 amending Directive 2001/18/EC as regards the possibility for the Member States to restrict or prohibit the cultivation of genetically modified organisms (GMOs) in their territory (OJ L 68, 13.3.2015, p. 1).
- Furgał-Dierżuk I., Strzetelski J., Twardowska M., Kwiatek K., Mazur M.** 2015. The effect of genetically modified feeds on productivity, milk composition, serum metabolite profiles and transfer of tDNA into milk of cows. *J. Anim. Feed Sci.* 24(1), 19–30. DOI: 10.22358/jafs/65649/2015.
- Gellrich K., Meyer H.H., Wiedemann S.** 2014. Composition of major proteins in cow milk differing in mean protein concentration during the first 155 days of lactation and the influence of season as well as short-term restricted feeding in early and mid-lactation. *Czech J. Anim. Sci.* 59(3), 97–106. DOI: 10.17221/7289-CJAS.
- Giraldo P.A., Shinozuka H., Spangenberg G.C., Cogan N.O., Smith K.F.** 2019. Safety assessment of genetically modified feed: is there any difference from food? *Front. Plant Sci.* 10, 1592. DOI: 10.3389/fpls.2019.0159.
- ISAAA.** 2017. Global status of commercialized biotech/GM crops in 2017: Biotech crop adoption surges as economic benefits accumulate in 22 years. ISAAA Brief No. 53. ISAAA: Ithaca, NY.
- IZ-INRA.** 2009. Normy żywienia przeżuwaczy [Standards for cattle nutrition], in: Wartość pokarmowa francuskich i krajowych pasz dla przeżuwaczy [Standards for ruminants nutrition]. Ed. J. Strzetelski. Kraków: Instytut Zootechniki–Państwowy Instytut Badawczy, 21–81 [in Polish].
- Kęsek-Woźniak M.M., Wojtas E., Zielak-Steciwo A.E.** 2020. Impact of SNPs in ACACA, SCD1, and DGAT1 genes on fatty acid profile in bovine milk with regard to lactation phases. *Animals* 10, 997. DOI: 10.3390/ani10060997.

- Maxhuni S., Halil K.** 2015. The factors importance to economization produced cheese mozzarella from cow's milk. *Res. Inv. Int. J. Eng. Sci.* 5(6), 29–35.
- Mullins E., Bresson J.L., Dalmay T., Dewhurst I.C., Epstein M.M., Firbank L.G., Guerche P., Hejatkó J., Moreno F.J., Naegeli H., Nogue G., Rostoks N., Serrano J.J., Savoini G., Veromann E., Veronesi F., Dumont A.F., Ardizzone M.** 2023. Animal dietary exposure in the risk assessment of feed derived from genetically modified plants. *EFSA J.* 21(1), 7732. DOI: 10.2903/j.efsa.2023.7732.
- Raport.** 2012. Wpływ pasz GMO na produktywność i zdrowotność zwierząt, transfer transgenicznego DNA w przewodzie pokarmowym oraz jego retencję w tkankach i produktach żywnościowych pochodzenia zwierzęcego, http://www.izoo.krakow.pl/zalaczniki/wazne_informacje/Wplyw_pasz_GMO_na_produkcyjnosć_i_zdrowotnosć_zwierzat.pdf, access 06.12.2019 [in Polish].
- Rodríguez-Bermúdez R., Fouz R., Rico M., Camino F., Souza T.K., Miranda M., Diéguez F.J.** 2023. Factors affecting fatty acid composition of Holstein cow's milk. *Animals* 13(4), 574. DOI: 10.3390/ani13040574.
- Shen C., Yin X., Jiao B., Li J., Jia P., Zhang X.-W., Cheng X.-H., Ren J.-X., Lan H.-D., Hou W.-B., Fang M., Li X., Fei Y.T., Robinson N., Liu J.-P.** 2022. Evaluation of adverse effects/events of genetically modified food consumption: a systematic review of animal and human studies. *Env. Sci. Eur.* 34(8). DOI: 10.1186/s12302-021-00578-9.
- Spek J.W., Dijkstra J., van Duinkerken G., Bannink A.** 2013. A review of factors influencing milk urea concentration and its relationship with urinary urea excretion in lactating dairy cattle. *J. Agric. Sci.* 151(3), 407–423.
- Sung H.G., Min D.M., Kim D.K., Li D.Y., Kim H.J., Upadhaya S.D., Ha J.K.** 2006. Influence of transgenic corn on the in vitro rumen microbial fermentation. *Asian-australas. J. Anim. Sci.* 19(12), 1761–1768. DOI: 10.5713/ajas.2006.1761.
- Vigolo V., Visentin E., Ballancin E., Lopez-Villalobos N., Penasa M., Marchi M.** 2023. β -casein A1 and A2: effects of polymorphism on the cheese-making process. *J. Dairy Sci.* 106(8), 5276–5287. DOI: 10.3168/jds.2022-23072.
- Výrostková J., Regecová I., Zigo F., Semjon B., Gregová G.** 2021. Antimicrobial resistance of *Staphylococcus* sp. isolated from cheeses. *Animals* 2021, 12(1), 36. DOI: 10.3390/ani12010036.
- Wiedemann S., Gürtler P., Albrecht C.** 2007. Effect of feeding cows genetically modified maize on the bacterial community in the bovine rumen. *Appl. Environ. Microbiol.* 73(24), 8012–8017. DOI: 10.1128/AEM.01060-06.
- Zicarelli L.** 2004. Buffalo milk: its properties, dairy yield and mozzarella production. *Vet. Res. Commun.* 28(1), 127–135. DOI: 10.1023/b:verc.0000045390.81982.4d.
- Zielak-Steciwko A.E., Kęsek M., Pecka-Kiełb E.** 2017. Share of selected protein fractions in the milk of Polish Holstein-Friesian cows depending on age, performance and stage of lactation. *Acta Sci. Pol. Zootechnica* 16(3), 3–10. DOI: 10.21005/asp.2017.16.3.01.

SKŁAD MLEKA KRÓW W ZALEŻNOŚCI OD WIEKU, FAZY LAKTACJI I PASZY GENETYCZNIE MODYFIKOWANEJ

Streszczenie. Celem pracy była analiza zmian składu mleka w zależności od obecności w paszy składników genetycznie modyfikowanych (GM) oraz w zależności od wieku krów i fazy laktacji. Badania przeprowadzono na stadzie bydła rasy polskiej holsztyńsko-fryzyjskiej odmiany czarno-białej (n = 50),

utrzymywanego w systemie uwięziowym. Paszą genetycznie modyfikowaną była mieszanka uzupełniająca. Na podstawie raportów wynikowych próbnich udojów zebrano dane o składzie ogólnym mleka, liczbie komórek somatycznych oraz zawartości mocznika. Uzyskane wyniki analiz podzielono na grupy: mleko krów pierwiastek (P) oraz będących w II i III laktacji (W). W każdej grupie wiekowej wyodrębniono po dwie podgrupy: krowy od 1. do 4. miesiąca laktacji (I) oraz krowy od 5. do 8. miesiąca laktacji (II). Powyższe podgrupy podzielono na mleko krów żywionych GMO (G) oraz bez GMO (B). Wykazano, że zastosowanie paszy z dodatkiem roślin genetycznie modyfikowanych wpływa na obniżenie poziomu kazeiny i wzrost laktozy w mleku. Zastosowanie paszy z produktów GMO nie wpłynęło na poziom tłuszczu, białka ogólnego, udziału tłuszczu do białka, poziom mocznika, liczbę komórek somatycznych w mleku oraz wydajność krów. Analizując uzyskane wyniki, można stwierdzić, że wycofanie z dawki pokarmowej dodatków paszowych na bazie pasz genetycznie modyfikowanych nie wpływa negatywnie na jakość technologiczną mleka.

Słowa kluczowe: pasza genetycznie modyfikowana, skład mleka, wiek krów, faza laktacji, bydlę mleczne.