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Comparison of fatty acid content of cows milk consuming different grass diets

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A b s t r a c t: The aim of this study was to evaluate effects of three dairy cow groups consuming different grass diets (Diet A comprising of 20% grass, Diet B comprising of 50% grass and 100% grazed grass-G) on cow milk proximate and fatty acid (FA) composition. The first principal component (PC1) accounted for 55.1%, and the second (PC2) accounted for 19.5% of the variance. The score values for PC1 and PC2 of the FAs show that milk fat from grass (MF G) was characterized by high C6:0, C8:0 and C14:0 contents. Milk from Diet A (MF A) was characterized by a higher content of C16:0. Milk from Diet B (MF B) was characterized by higher contents of C18:1n-9 and C18:2n-6 than milk from Diet A. The most favorable FA composition was in milk from Diet B, comprising 50% grass. The least favorable FA composition was in milk from Diet A, comprising 20% grass and in milk from 100% grazed grass. However, more testing is needed to bring a conclusion which food for dairy cows is the best.

Keywords: *chemical composition, feed composition, milk fatty acids, principal component analysis.*

Introduction

Diets consumed by lactating dairy cows are low in fat content, generally containing only about 4–5% lipid (*Lock and Baumann, 2004*). Butyric (C4:0) to myristic acids (C14:0) are generated through *de novo* synthesis in the mammary gland, while varying amounts of palmitic acid (C16:0) are derived from *de novo* synthesis and from the uptake of circulating lipids (*Grummer* 1991*; Sejrsen et al.,* 2007*; Neville and Picciano,* 1997). The main isomer present in milk is the *trans* monounsaturated fatty acid (MUFA), vaccenic acid (18:1trans-11) (*Chillard et al*., 2007). In the mammary gland, the fatty acids (FAs) undergo desaturation by biohydrogenation of linoleic acid from the rumen to rumenic acid (RA, CLA cis-9, trans-11), which finally converts C18:1 trans-11 to stearic acid (C18:0) (*Harfoot and Hazlewood*, 1997). Conjugated linoleic acid (CLA) is common in milk, and is a mixture of positional and geometric isomers of linoleic acid (C18:2n-6) with conjugated double bonds (*Bauman and Lock*, 2006). The high natural levels in ruminant depot fat originate partly from bacteria in the rumen (*Harfoot and Hazlewood*, 1997). The anticarcinogenic, antidiabetogenic, anti-atherogenic and immunomodulatory effects of CLA have been clearly established (*Banni et al.,* 2003; *Belury,* 2002; *Ip et al*., 2003; *Lee et al*., 2004; *Pariza et al*., 1996). The predominant source of CLA in human diets is ruminant-derived food products, with dairy products contributing CLAs in various isomers but predominantly as rumenic acid. Although CLA occurs in small amounts in vegetable oils, the meat and milk of ruminants contain particularly high concentrations, varying between 0.5% and 2% of total lipids (*Bauman and Griinari*, 2003; *Jenkins et al*., 2008; *Parodi*, 2003). CLA is a component of milk fat, and hence, research has concentrated on increasing the CLA content per unit of fat. Processing has little effect on CLA, so the content in food products is related to the CLA concentration in the starting fat (*Parodi*, 2003). These are the reasons for the intense interest in the distribution, synthesis, and concentration of CLA in foods that is believed to be health-promoting for consumers. Linolenic acid (C18:3n-3) is derived principally from forage crops, being a major component of the oilseeds and concentrates that are fed to dairy cows (*Lock and Baumann*, 2004). *Gaspardo et al.* (2010) found unsaturated FAs and long-chain C18 FAs can be used as efficient markers for the discrimination of milk based on country of origin. This is in agreement with the findings of other authors who pointed out that the variation of FA compositions in milk can be related to the

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origin of the animals and breed (*Poulsen et al*., 2012; *Palladino et al*., 2010). The aim of this study was to evaluate effects of three cow grass-based diets on cow milk proximate and fatty acid composition.

Material and Methods

Compliance with ethical standards

The experimental use of animals and procedures for their management was performed in compliance with the Animal Welfare Law, Serbia, and approved by the Ethics Committee, Institute of Meat Hygiene and Technology, Belgrade.

Dairy cow feeding and milking

A total of 21 lactating Holstein-Friesian dairy cows were divided into three groups and then each group was assigned to one of three dietary treatments. Animal groups were fed one of three experimental diets (Diet A comprising of 20% grass, Diet B comprising of 50% grass, both of which were mixed diets, and grazed grass G). Cows were milked twice daily and individual milk yields were recorded at each milking using the afimilk (Kibbutz Afikim, Israel) system. Cows were fed at least 35 days before each sampling.

Milk and feed analysis

Milk samples were cooled and transported to the laboratory for analysis of fat and protein contents. The fat content was measured according to the Gerber butyrometric method (ISO 488:2008), the protein content was measured using a fully automated Kjedahl analyser (Kjeltec 8400, Foss, Hillerød, Denmark)

Feed samples were analyzed for moisture (*ISO* 6496:1999), crude protein, total fat (*ISO* 6492:1999) crude ash (*ISO* 5984:2002) and fibre content (*ISO* 6865:2000). The protein content was measured using a fully automated Kjedahl analyser (Kjeltec 8400, Foss, Hillerød, Denmark). Nitrogen-free extractives (NFE) as a measure of the soluble carbohydrates in the feed, such as percentage of starch and sugar, were calculated.

FA analysis by capillary gas chromatography

The FA composition was determined by capillary gas chromatograph previously using accelerated solvent extraction (ASE), (ASE 200, Dionex, Sunnyvale, CA, USA) with petroleum ether and isopropanol mixture (60:40, v/v) at 100°C over three static cycles of 1 min under nitrogen at 12 MPa. The solvent from the collected extracts was removed under a stream of nitrogen (Dionex Solvent evaporator 500, Dionex, Sunnyvale, CA, USA) at 50°C until dry. The fatty acid methyl esters (FAMEs) were prepared by the method of base catalyzed methylation of FAs with sodium methoxide in methanol according to the method proposed by *Christie et al.* (2001). FAMEs were determined by gas-liquid chromatography (Shimadzu 2010, Kyoto, Japan) with with flame ionization detector (FID) on HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm). Injector and detector temperature were 250°C and 280°C, respectively. Nitrogen was used as the carrier gas at flow rate of 1.87 mL min^{-1}. The injector split ratio was set at 1:50. The injected volume was 1 μL. Detector gases: hydrogen 40 mL min^{-1}, synthetic air 400 mL min^{-1}, make-up gas (nitrogen) 30 mL min^{-1}. Temperature program for column: 50°C, hold 1 min; at a rate of 13°C min^{-1} to 175°C, hold 15 min; at a rate of 4°C min^{-1} to 215°C, hold 10 min; at a rate of 2°C min^{-1} to 230°C, hold 5 min. Total analysis time was 61.5 min. The chromatographic peaks in the samples were identified by comparing FAME peaks with peaks in FAME mix standard (Supelco 37 Supelco, Bellefonte, PA) and to which a mixture of 5 mg ml^{-1} CLA was added (mixture of methyl cis 9,11- and trans-10,12-octadecadienoic acid, O5632, Sigma Aldrich). Each milk sample was analyzed in triplicate.

Statistical analysis

All chemical analyses were performed in three replicates and the results were statistically analyzed. One factor analysis of variance (ANOVA) was used to compare grouped data. Tukey-Kramer test was used to test the significance of differences between the observed means. All statistical analyses as well as principal component analysis (PCA) were conducted using JMP 10 software (SAS Institute Inc.USA).

Results and Disccusion

Milk production and diets composition

The chemical composition of the milks is given in Table 1.

There was no significant difference in weight of cows fed different diets. There was a significant difference in the protein content of the milks, the highest being in milks from Diets A and B and the lowest in milk from grazing ($P < 0.05$). There were

Table 1. The effect of dietary treatment on dairy cow and milk performance

	MF (A) (n = 6)	MF (B) (n = 6)	MF (G) (n = 9)	P- value
Milk yield, kg day^{-1}	22	28	25	NS
Weight, kg cow^{-1}	597±8^{NS}	560±8^{NS}	550±4^{NS}	NS
Protein, %	3.41±0.01^{A}	3.21±0.02^{AB}	2.90±0.01^{B}	**
Fat, %	3.60±0.01^{NS}	4.18±0.03^{NS}	3.75±0.01^{NS}	NS

Legend: Values are mean ± SEM, n – number of samples. P-value – level of significance; NS – not significant, ** Means within a row with different superscripts differ significantly (P < 0.01); MF (A) – milk from diet A; MF (B) – milk from diet B; MF (G) – milk from grazing

no significant differences observed in milk fat content (P > 0.05). However, significant different for protein content and fat content were observed in study of *Palmar et al*. (2020).

Chemical and FA composition of the diets are given in Table 2.

Diet A and Diet B contained similar contents of crude proteins, total fat and crude ash but differed in moisture, crude fibre and in nitrogen-free extractives; the latter were highest in Diet A. Silage accounted for 50% of Diet A and 20% of Diet B. Also, the FA composition of Diets A and B differed. Diet B contained higher levels of saturated FAs (SFAs), including palmitic acid (C16:0) and stearic acid (C18:0), than Diet A. Higher levels of monounsaturated FAs (MUFAs) occurred in Diet A, among which oleic acid (C18:1n-9) was also higher than in Diet B. Diet A contained higher levels of polyunsaturated FAs (PUFAs), of which linoleic acid (C18:2n-6) was higher than in Diet B, but linolenic

Table 2. Chemical and fatty acid composition in diets (%)

Proximate composition	Diet A	Diet B	Diet G
Crude proteins	8.10	9.49	3.86
Moisture	42.07	35.99	80.72
Crude total fat	1.66	1.42	0.77
Crude ash	4.61	5.19	1.95
Crude fibre	7.61	22.39	2.65
NFE	35.96	25.52	10.05
Silage	50	20	0
Concentrate	30	30	0
Grass	20	50	100
Fatty acid composition			
C16:0	18.02	23.32	28.05
C18:0	2.61	4.99	6.45
C18:1n-9	25.67	20.18	13.68
C18:2n-6	47.36	37.92	13.34
C18:3n-3	4.02	10.51	32.38
SFA	21.62	31.10	36.54
MUFA	25.93	20.47	17.24
PUFA	51.38	48.43	46.17

Legend: NFE – nitrogen-free extractives; SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

acid (C18:3n-3) was higher in Diet B than in Diet A. Grass was full of moisture, nitrogen-free extractives and linolenic acid.

Milk fatty acid composition

Milk FA composition is presented in Table 3.

SFAs were the most abundant FA class, being statistically highest in milk from Diet A and milk from grazing and the lowest in milk from Diet B. MUFAs were the next most abundant FA class, being statistically highest in milk from Diet B and the lowest in milk from Diet A and milk from grazing. PUFAs were statistically highest in milk from Diet B followed by milk from grazing and were the lowest in milk from Diet A ($P < 0.05$). Among the SFAs, palmitic acid was present in the greatest amounts, and was statistically highest in milk from Diet A and statistically lowest in milk from Diet B ($P < 0.05$). Among the MUFAs, oleic acid was statistically highest in milk from Diet B followed by milk from grazing and was the lowest in milk from Diet A ($P < 0.05$). Among the PUFAs, linoleic acid was statistically highest in milk from Diet B, followed by milk from grazing and was the lowest in milk from Diet A ($P < 0.05$). The profile of *c*9*t*11-CLA isomers

Table 3. Effects of dietary treatments on milk fatty acid profiles (% of total FA).

FAs	MF (A) (n = 6)	MF (B) (n = 6)	MF (G) (n = 9)	P- value
C4:0	2.44±0.02^{B}	2.47±0.02^{B}	3.96±0.02^{A}	**
C6:0	1.87±0.01^{B}	1.60±0.02^{C}	2.34±0.02^{A}	***
C8:0	1.15±0.01^{B}	0.97±0.01^{B}	1.33±0.02^{A}	**
C10:0	2.76±0.04^{AB}	2.26±0.05^{B}	3.17±0.06^{A}	**
C12:0	3.31±0.06	2.48±0.07	3.52±0.09	NS
C14:0	13.52±0.08^{A}	9.67±0.10^{B}	12.90±0.12^{A}	**
C16:0	42.01±0.23^{A}	29.67±0.22^{C}	34.30±0.30^{B}	***
C16:1	1.44±0.02^{A}	1.64±0.04^{A}	0.89±0.01^{B}	**
C17:0	0.53±0.01^{B}	0.67±0.01^{A}	0.55±0.01^{B}	**
C18:0	8.39±0.12^{B}	10.95±0.20^{AB}	11.02±0.25^{A}	**
C18:1 trans-11	1.49±0.05	2.46±0.15	1.46±0.08	NS
C18:1n-9	19.81±0.28^{B}	33.58±0.02^{A}	20.39±0.27^{B}	**
C18:2n-6	1.40±0.01^{C}	2.42±0.02^{A}	1.88±0.03^{B}	***
C20:0+C18:3n-6	0.23±0.01	0.25±0.01	0.28±0.01	NS
c9,t11 CLA	0.15±0.01^{AB}	0.10±0.01^{B}	0.17±0.01^{A}	**
C20:4n-6	0.10±0.01^{B}	0.16±0.01^{AB}	0.16±0.01^{A}	**
SFA	77.10±0.29^{A}	61.90±0.09^{B}	74.75±0.33^{A}	**
MUFA	21.25±0.26^{B}	35.23±0.04^{A}	21.28±0.27^{B}	**
PUFA	1.65±0.05^{C}	2.67±0.02^{A}	2.21±0.03^{B}	***
SCFA	8.26±0.05^{B}	7.30±0.05^{B}	10.88±0.10^{A}	**
MCFA	61.87±0.37^{A}	45.20±0.24^{C}	53.82±0.49^{B}	***
LCFA	31.09±0.46^{B}	49.41±0.16^{A}	34.75±0.56^{B}	**
VLCFA	0.51±0.02	0.58±0.01	0.62±0.01	NS

Legend: Values represent mean ± SEM, n – number of samples; P-value –level of significance; NS- not significant, ** Means within a row with different superscripts differ significantly ($P < 0.01$); *** Means within a row with different superscripts differ significantly ($P < 0.001$); SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids, SCFA – short-chain fatty acids (< C11:0); MCFA – medium-chain fatty acids (C12:0-C17:0); LCFA – long-chain fatty acids (C18-C19); VLCFA – very long-chain fatty acids (> C19:0); MF (A) – milk from diet A; MF (B) – milk from diet B; MF (G) – milk from grazing.

in the milks was statistically highest in milk from grazing, followed by milk from Diet A and was the lowest in milk from Diet B ($P < 0.05$) (Table 3). The same profile was obtained in study of *Trbović et al.* (2017). The characteristic FA profile of milk from the grazing dairy cows was predominantly C16:0, C18:0 and C18:1n-9. The FAs of milk from Diet A were high in C16:0, and the FAs of milk from grazing and Diet B and C18:1n-9 were high in milk from Diet B.

With our grass-based diets, the short-chain FAs (SCFAs) composed 7-11% of the cow milk FAs across all dietary groups. Conversely, if lipid dietary supplements are rich in medium-chain FAs (MCFAs), this could account for the different MCFA content of 45% in Diet B milk and 62% in Diet A milk; in fact, this is likely a consequence of the C16:0 content (29.67% to 42.01% in milks from Diet B and A, respectively). Long-chain FAs (LCFAs) composed 31–49% of cow milk FAs and, in contrast to MCFA, were the lowest in milk from Diet A, followed by milk from grazing, but were the highest in milk from Diet B. Very long-chain FAs (VLCFA) were very similar in the three milk groups and did not differ statistically. In contrast to short SCFA, very little VLCFA is synthesized *de novo* by ruminants and therefore most VLCFA must be ingested in the feed if these moieties are to be present in the milk (*Elgersma et al.*, 2006; *Chilliard et al.*, 2007). LCFA in milk originate almost exclusively from the

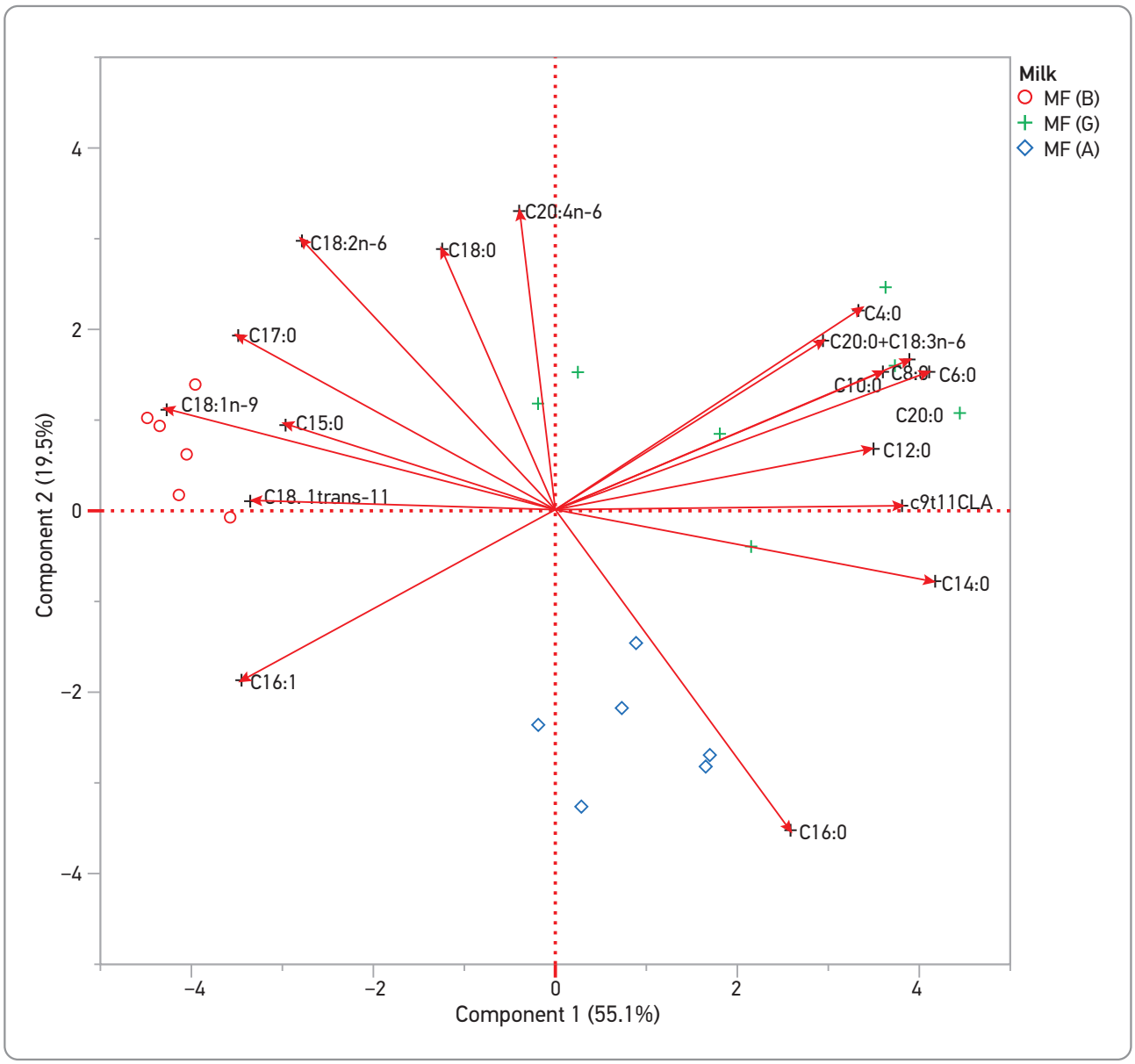

Figure 1. Principal component analysis among milk fatty acids (FA) (% of total fatty acids) in milk from 3 different diets

feed, but can be considerably modified in the rumen. Within the rumen, isomerization and hydrogenation depend on the FA content in the feed, but they also relate to the amounts of feed-derived starch and fiber that reach the rumen. According to *Chillard et al.* (2007), the potential to decrease MCFA in milk *via* cow diet is considerable, as occured in our study. For example, in milk from the grazed grass diet, MCFA composed 54% of the cow milk FAs, in milk from Diet A, MCFA accounted for a higher percentage of milk FAs, (62%) and in milk from Diet B, the amount of MCFA was lower (45%). This was due to the different cow diets. In contrast, our three cow diets had no effect on concentrations of SCFA in cow milk fat, as was observed by *Chillard et al.* (2007). The enzyme Δ9-desaturase catalyzes the introduction of a cis-double bond mainly favoring the conversions of C16:0 into C16:1 and C18:0 into C18:1 n-9 (*Ntambi and Miyzaki*, 2004; *Bauman et al*., 2006; *Jenkins et al*., 2008), as obtained in our study (Table 3). According to *Poulsen et al*. (2012), the results obtained in the current study show that grass induces higher C6:0 to C14:0 levels in milk which could be related to reduced *de novo* synthesis of FA. C18:3n-3 probably was derived from grass, which accounted for 50% of Diet B and 100% of the grazing diet.

PCA performed on the FAs (expressed as a percentage of the total FA) in the 21 milk samples provided better insight into the data structure (Figure 1). The analysis resulted in a two-principal-component model that explained 74.6% of the total variance. The first principal component (PC1) accounted for 55.1%, and the second (PC2) accounted for 19.5% of the variance. The score values for the first two principal components (PC1 and PC2) of the FAs expressed as percentages of the total FAs show that milk fat from grass (MF G) was characterized by high C6:0, C8:0 and C14:0 contents. Milk from Diet A (MF A) was characterized by a higher content of C16:0. Milk from Diet B (MF B) was characterized by higher contents of C18:1n-9 and C18:2n-6 than milk from Diet A.

Conclusion

In this study, we examined the proximate and FA composition in Holstein-Friesian dairy cows fed on three dietary treatments. There was a significant difference in the protein content of the milks, the highest being in milks from Diets A and B and the lowest in milk from grazing. There were no significant differences observed in milk fat content. Diet B and Diet A contained similar contents of proteins, total fat and crude ash but differed in moisture content, crude fibre content and in nitrogen-free extractives; the latter were highest in Diet A. The most favorable FA composition was in milk from Diet B, comprising 50% grass, 30% concentrate and 20% silage. The least favorable FA composition was in milk from Diet A, comprising 20% grass, 30% concentrate and 50% silage and in milk from 100% grazed grass. The different feeding regimens resulted in dietary responses in the dairy cows that significantly affected the milk fat composition. However, more testing is needed to bring a conclusion which food for dairy cows is the best.

Poređenje sadržaja masnih kiselina u kravljem mleku u zavisnosti od načina ishrane životinja

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A p s t r a k t: Cilj ispitivanja ovog rada je bio da se procene efekti tri različita načina ishrane mlečnih krava (ishrana A- od 20% trave, ishrana B - 50% trave i 100% ishrana na ispaši-G) na masnokiselinski sastav kravljeg mleka. Prva glavna komponenta (PC1) čini 55,1%, a druga (PC2) 19,5% varijanse. Vrednosti skora za PC1 i PC2 FA pokazuju da se mlečna mast (MF G) karakteriše visokim sadržajem C6:0, C8:0 i C14:0. Mleko krava koje su bile u A grupi (MF A) karakteriše veći sadržaj C16:0. Mleko krava iz B grupe (MF B) karakteriše veći sadržaj C18:1n-9 i C18:2n-6 u odnosu na mleko krava hranjenih u grupi A. Najpovoljniji masnokiselinski sastav bio je u mleku krava iz grupe B, sa 50% trave. Najnepovoljniji masnokiselinski sastav bio je u mleku krava iz grupe A, i u mleku krava hranjenih 100% na ispaši. Međutim, potrebno je veći broj istraživanja da bi se doneo zaključak koja je ishrana mlečnih krava najbolja.

Ključne reči: *hemijski sastav, sastav hrane za životinje, masne kiseline mleka, analiza glavnih komponenti.*

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