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EFFECT OF HUMATES IN DIET OF DAIRY COWS ON THE RAW MILK MAIN COMPONENTS

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ABSTRACT

The effect of supplemental humic substances (HS) on the main milk components was investigated. A total of 10 dairy cows (Czech pied cattle, crossbred Czech pied cattle × Ayrshire and crossbred Czech pied cattle × Red Holstein) were tested. Animals were randomly divided into 2 groups, control (C) and experimental (E). Animals fed the same feed mixture and group E was additionally supplemented with HS (200 mg.kg^{-1} of product Humafit prepared from the Sakhalin Leonardite). The experimental period took 3 months. Cows were milked twice a day. The milk composition (lactose, fat, crude protein, pure protein and casein) of every cow was monitored on days 0, 14, 28, 42, 56, 70 and 84 of the experiment. Pure protein content was determined by Kjeldahl method, other components were analysed using an infrared analyserMilkoScan FT 120. It was found that the crude protein, pure protein and casein content in milk of group E significantly (p <0.05) increased from the 56th day of the experimental period. Differences of the protein fraction contents in group C and of the dry matter, non-fat dry matter, lactose and fat content in both groups were non-significant (p <0.05).Higher protein and especially casein content in milk could be very important for the cheesemaking as it could increase the cheese yield.

Keywords: humic substances; Sakhalin Leonardite; dairy cows; milk proteins; casein

INTRODUCTION

Humates are formed from chemical and biological decomposition of plant, animal and microbial materials mostly by soil bacteria. As high-molecular heteropolycondensed compounds with colloidal or amorphous nature and yellow to brown-black colour they contain variable functional groups such as amide, amine, carbonyl, carboxyl, hydroxyl, phenol or sulphhydryl. Humic substances (HS) are the main organic component of soil, peat, different types of coal, lignite, fresh and sea water, sewage and their sediments. Humates principal constituents are humus, humic acid, fulvic acid, ulmic acid and some trace microelements for instance copper, iron, manganese and zinc **(Visser, 1973; Stevenson, 1994)**. They are currently used in industry, environmental and bio-medicine and agriculture **(Cunha et al., 2014; Rose et al., 2014)**.

HS have been started to investigate in some areas of animal husbandry respective health, wellbeing and production during the past few decades. The HS have been reported to have significant analgesic, antidiarrheal, anti-inflammatory, antimicrobial, antiseptic, antitoxic, antiviral and immunostimulatory properties. They have been reported to have stimulating effects on oxygen transport, form protective film on the mucosa of gastrointestinal tract and ensure an improved nutrient utilization in animal feed **(Islam et al., 2005; Kucukersan et al., 2005; Trckova et al., 2005; Písaříková et al., 2010)**. These specific properties probably bring also possible benefits in animal production. Many authors indicated that supplemental humates reduced animal mortality, improved health, growth performance, feed conversion and some production characteristics of pigs **(Wang et al., 2008; Písaříková et al., 2010; Bai et al., 2013)**, poultry **(Hayirly et al., 2005; Šamudovská and Demeterová, 2010; Gładkowski et al., 2011)** and dairy cattle **(Degirmencioglu, 2012, 2014)**.

However, the use of the HS as a dietary supplement in dairy cow diet has not been well reported. Therefore, the aim of this research was to determine the effects of humates supplemented diet on the main milk components in dairy cows. The hypothesis was that the humates supplementation will affect the dairy cows' raw milk composition and the main milk components content will be increased.

MATERIAL AND METHODOLOGY

Humates characterisation

The humates (product Humafit) used in the experiment were obtained from ReConsulting a.s. company, CZ. The Humafit was prepared from the Sakhalin Leonardite and according to the producer contained 4.20 g.100 g^{-1} of moisture, 95.80 g.100 g^{-1} of dry matter, 65.34 g.100 g^{-1} of natural humic acids, 7.74 g.100 g^{-1} of crude protein,

Table 1 Formula of feed mixture.

Ingredient	Content (g.100 g^{-1} of DM)
Alfalfa haylage	21.44
Corn silage	20.62
Corn meal	17.87
Meadow hay	7.83
Soybean meal	6.93
Barley grain	6.05
Malt meal	5.27
Wheat bran	4.83
Wheat grain	3.99
Rapeseed meal	2.92
Sugar beet pulps	0.96
Vitamin and mineral premix*	1.31

Note: DM = dry matter; *premix composition per 1 kg: 150 g Ca, 60 g P, 90 g Na, 80 g Mg, 2 g Fe, 2 g Cu, 8 g Mn, 10 g Zn, 0.04 g Se, 0.20 g I, 0.04 g Co, 0.02 g S, 1000 × 1000 IU vitamin A, 200 × 1000 IU vitamin D_3, 5.00 g vitamin E.

Table 2 Chemical composition of feed mixture.

Component	Content
DM (g.100 g^{-1})	46.13
Protein (g.100 g^{-1} of DM)	16.65
Fat (g.100 g^{-1} of DM)	2.58
Ash (g.100 g^{-1} of DM)	7.04
Starch (g.100 g^{-1} of DM)	19.52
Fiber (g.100 g^{-1} of DM)	17.46
NDF (g.100 g^{-1} of DM)	32.22
ADF (g.100 g^{-1} of DM)	17.34
NE_L (MJ.kg^{-1})	1.27

Note: DM = dry matter, NDF = neutral detergent fiber, ADF = acid detergent fiber, NE_L = net energy of lactation.

Table 3 MilcoScan FT 120 calibration coefficients.

Analyte	Slope	Intercept	B0-coef.
Dry matter (total solids)	1.0542	-0.6963	1.5476
Non-fat dry matter (solids non-fat)	1.0060	-0.1504	0.6522
Lactose	1.1905	-0.6819	0.6027
Fat	1.0153	-0.0093	0.3590
Crude protein	0.9919	0.0644	0.1440
Casein	1.0758	-0.3635	-0.9734

0.82 g.100 g^{-1} of fat, 21.90 g.100 g^{-1} of ash, 1.45 g.100 g^{-1} of nitrogen, 0.97 g.100 g^{-1} of phosphorus, 0.40 g.100 g^{-1} of calcium and 0.24 g.100 g^{-1} of sodium. Humic acids were characterised according to the **Novák and Hrabal (2011)** and **Madronová (2011)**. Other components were determined according to **AOAC (2012)**.

Animal care, experimental design, animals and diets

The experimental conditions were designed in accordance with the Guide for the care and use of agricultural animals in research and teaching **(FASS, 2010)**.

In the experiment Czech pied cattle, crossbred Czech pied cattle (79 – 87%) × Ayrshire (13 – 21%) and crossbred Czech pied cattle (83 – 87%) × Red Holstein (13 – 17%) dairy cows as research animals were used. A total of 10 dairy cows with balanced characteristics (body weight 654 ±48 kg, lactation period 97 ±21 days of milk production, producing 28.7 ±5.7 kg.day^{-1}) were randomly divided into 2 groups with the same breed distribution: control (C) and experimental (E). There were 5 animals per group. Animals were housed in a free stall, allowed *ad libitum* access to water and fed twice a day (at 5 : 30 and 15 : 00) with feed mixture (Tables 1 and 2) prepared according to the Nutrient Requirements of Dairy Cattle **(NRC, 2001)**.

The experimental group became extra supplemented by Humafit (ReConsulting a.s., CZ) which was manually handed to each experimental animal in dose of 200 mg.kg^{-1}. Cows were milked twice a day (at 5 and 17 hours) with an automated milking plant. The health status of the animals was checked before each milking to avoid milk affected by mastitis. The experimental period took 3 months. The seasonal effect of components were not taken into account because the samples from the both groups (control and experimental) were taken in parallel at the same time.

Milk sampling

Daily raw milk samples (500 mL, 1 : 1 from two daily milking) were taken from each cow and collected on days 0, 14, 28, 42, 56, 70 and 84 of the experimental period. Samples were preserved using Broad Spectrum Microtabs II (Advanced Instruments, Inc., USA) containing 8 mg of Bronopol and 0.30 mg of Natamycin. These tablets inhibit bacteria, yeasts and molds, provide extended shelf life and reduce lipolysis of milk samples. After this preservation the samples were stored at 4 °C until analysis, which was performed the next day after milk sampling.

Analytical methods

The raw milk samples were analysed for dry matter (total solids), non-fat dry matter (solids non-fat), lactose, fat, crude protein and casein content using an infrared automatic milk analyser MilkoScan^{TM} FT 120 (FOSS Electric A/S, DK) according to **ISO 9622**. The MilkoScan calibration coefficients (slope, intercept and and B0-coef for filters) for these analytes are in Table 3. Pure protein content was determined by the Kjeldahl method **(ISO 8968-1, ISO 8968-3, ISO 8968-5)**. All measurements were performed twice for each sample.

Statistical analysis

The outliers were removed from the obtained data by Grubbs' test on the level of significance α = 0.05 using Microsoft Excel 2003 (Microsoft Office Excel 2003, Microsoft Corporation, USA). Results in form of arithmetic means from 10 parallel measurements with standard deviation are expressed as difference from the day 0 in order to minimise the effect of genotype, animal individuality and stage of lactation. The one-way ANOVA F-test on the level of significance α = 0.05 of the dry matter, non-fat dry matter, lactose, fat, crude protein, pure protein and casein content was performed by Microsoft Excel 2003 (Microsoft Office Excel 2003, Microsoft Corporation, USA). Means followed by the same letters have the same statistical significance.

RESULTS AND DISCUSSION

Effects of supplemental humates in form of the product Humafit on the main milk components content were investigated in dairy cows. Both groups of animals, C (control) and E (experimental), were fed with feed mixture described in Tables 1 and 2. Group E was daily supplemented with 200 mg.kg^{-1} of Humafit. The initial milk composition (dry matter, non-fat dry matter, lactose, fat, crude protein, pure protein and casein content respectively) and also changes of the main milk component content during the experimental period are shown in Tables 4 to 10. The contents of the main milk components were in line with **Bujko et al. (2011)**, **Filipejová et al. (2011)** and **Zajác et al. (2012, 2015)**.

No significant differences (p < 0.05) were observed in dry matter, non-fat dry matter, lactose and fat content in milk of both animal groups during the experiment. The same trend was recorded for the content of crude protein, pure protein and casein in group C (p < 0.05) milk. On the contrary, the crude protein, pure protein and casein content in milk of cows from group E supplemented with humates were significantly (p < 0.05) higher from the 56th day of the experimental period.

HS have been recognised to form a protective film on the gastrointestinal mucosa and positively modulate the gastrointestinal processes as so as nutrient utilization **(Lange et al., 1996; Islam et al., 2005; Písaříková et al., 2010)**. Their antimicrobial, antiviral, antiseptic, anti-inflammatory, analgesic and immunostimulatory properties have been also well-reported **(Lange et al., 1996; Islam et al., 2005; Kucukersan et al., 2005; Agazzi et al., 2007)**. Other authors demonstrated beneficial influence of humates on growth performance, feed efficiency and feed conversion ratio in the livestock **(Hayirly et al., 2005; Avci et al., 2007; Wang et al., 2008; Šamudovská, and Demeterová, 2010; Bai et al., 2013)**, on meat quality in pigs **(Wang et al., 2008; Bai et al., 2013)** and on egg production and fatty acid profile of egg yolk modulation in hens **(Hayirly et al., 2005; Gładkowski et al., 2011)**.

Degirmencioglu (2012, 2014) focused on the effects of different levels of humic acid (HA) supplementation (0, 1 and 3 g HA.kg^{-1}) on blood characteristics, milk yield and milk composition in dairy goats. He reported significantly lower levels of total and LDL cholesterol after HA supplementation. However, results of milk yields were inconsistent and he did not observe improvements in milk composition respectively non-fat dry matter, lactose, fat and protein content **(Degirmencioglu, 2012, 2014)**.

Table 4 Effect of supplemental humic substances on dry matter content.

Time (days)	Dry matter content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	12.75 ±0.79	1.00	12.85 ±0.81	1.00
14	12.49 ±1.55	0.98[a]	12.22 ±0.43	0.96[a]
28	12.69 ±1.65	0.99[a]	12.97 ±0.63	1.01[a]
42	12.92 ±0.88	1.01[a]	13.13 ±0.76	1.02[a]
56	12.68 ±0.99	1.00[a]	13.24 ±1.17	1.03[a]
70	13.23 ±0.65	1.04[a]	12.89 ±0.43	1.01[a]
84	13.18 ±0.84	1.04[a]	13.74 ±0.87	1.07[a]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period.

Table 5 Effect of supplemental humic substances on non-fat dry matter content

Time (days)	Non-fat dry matter content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	8.50 ± .60	1.00	8.45 ±0.76	1.00
14	8.72 ±1.49	1.02[a]	8.49 ±0.42	1.01[a]
28	8.32 ±1.35	0.98[a]	8.73 ±0.80	1.05[a]
42	8.56 ±0.63	1.01[a]	8.56 ±0.50	1.02[a]
56	8.42 ±0.40	1.00[a]	8.96 ±1.26	1.06[a]
70	9.20 ±0.63	1.09[a]	8.80 ±0.53	1.05[a]
84	8.88 ±0.78	1.04[a]	9.04 ±0.76	1.08[a]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period.

Table 6 Effect of supplemental humic substances on lactose content.

Time (days)	Lactose content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	4.93 ±0.13	1.00	4.95 ±0.15	1.00
14	4.78 ±0.09	0.97[b]	4.80 ±0.14	0.97[a]
28	4.78 ±0.16	0.97[b]	5.00 ±0.11	1.01[a]
42	4.83 ±0.17	0.98[a]	4.95 ±0.10	1.00[a]
56	4.78 ±0.23	0.97[a]	5.10 ±0.13	1.03[a]
70	4.88 ±0.18	0.99[a]	4.90 ±0.06	0.99[a]
84	4.98 ±0.22	1.01[a]	4.80 ±0.20	0.97[a]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period. [b]Significant difference (p <0.05) from day 0 of the experimental period.

Table 7 Effect of supplemental humic substances on fat content

Time (days)	Fat content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	4.29 ±0.52	1.00	4.61 ±0.76	1.00
14	3.99 ± 0.58	0.93[a]	4.15 ±0.29	0.90[a]
28	4.33 ±0.84	1.01[a]	4.38 ±0.15	0.95[a]
42	4.72 ±0.62	1.10[a]	4.75 ±1.01	1.03[a]
56	4.33 ±0.78	1.01[a]	4.89 ±0.78	1.06[a]
70	4.55 ±0.58	1.06[a]	4.56 ±0.27	0.99[a]
84	4.76 ±0.44	1.11[a]	5.16 ±0.42	1.12[a]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period.

Table 8 Effect of supplemental humic substances on crude protein content

Time (days)	Crude protein content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	3.48 ±0.38	1.00	3.21 ±0.13	1.00
14	3.44 ±0.42	0.99[a]	3.21 ±0.09	1.00[a]
28	3.51 ±0.41	1.01[a]	3.37 ±0.08	1.05[a]
42	3.55 ±0.40	1.02[a]	3.40 ±0.09	1.06[a]
56	3.51 ±0.30	1.01[a]	3.50 ±0.14	1.09[b]
70	3.51 ±0.39	1.01[a]	3.69 ±0.13	1.15[b]
84	3.55 ±0.16	1.02[a]	3.88 ±0.19	1.21[b]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period. [b]Significant difference (p <0.05) from day 0 of the experimental period.

Table 9 Effect of supplemental humic substances on pure protein content

Time (days)	Pure protein content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	3.17 ±0.28	1.00	3.09 ±0.10	1.00
14	3.20 ±0.27	1.01[a]	3.06 ±0.10	0.99[a]
28	3.26 ±0.31	1.03[a]	3.21 ±0.06	1.04[a]
42	3.23 ±0.27	1.02[a]	3.24 ±0.07	1.05[a]
56	3.11 ±0.25	0.98[a]	3.34 ±0.07	1.08[b]
70	3.11 ±0.13	0.98[a]	3.49 ±0.10	1.13[b]
84	3.07±0.06	0.97[a]	3.58 ±0.13	1.16[b]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period. [b]Significant difference (p <0.05) from day 0 of the experimental period.

Table 10 Effect of supplemental humic substances on casein content.

Time (days)	Casein content			
	Control group		Experimental group	
	(g.100 g^{-1} ±SD)	RV (day/day 0)	(g.100 g^{-1} ±SD)	RV (day/day 0)
0	2.68 ±0.29	1.00	2.47 ±0.10	1.00
14	2.57 ±0.38	0.96[a]	2.42 ±0.15	0.98[a]
28	2.65 ±0.34	0.99[a]	2.62 ±0.12	1.06[a]
42	2.79 ±0.31	1.04[a]	2.59 ±0.11	1.05[a]
56	2.73 ±0.22	1.02[a]	2.67 ±0.10	1.08[b]
70	2.68 ±0.30	1.00[a]	2.72 ±0.11	1.10[b]
84	2.79 ±0.09	1.04[a]	2.96 ±0.16	1.20[b]

Note: SD = standard deviation, RV = relative value. Results are expressed as arithmetic mean of four parallel evaluations. [a]No significant differences (p >0.05) from day 0 of the experimental period. [b]Significant difference (p <0.05) from day 0 of the experimental period.

Our obtained data related to the milk composition are in agreement with average milk composition analysed in Central Milk Laboratory of the Czech Republic **(CMDA, 2013; Kouřimská et al., 2014)**. Data related to the dry matter content, non-fat dry matter content, lactose and fat are in agreement with **Degirmencioglu (2012, 2014)**. On the contrary, we recorded significantly higher crude protein, pure protein and casein content after the 56th days of the humates addition. These results could be attributable principally to the different HS preparations, animal species and ages and experimental conditions (dose of humates, longer length of experimental period) as was described preliminary **(Wang et al., 2008)**.

CONCLUSION

According to the current experiment results it could be concluded that dietary supplementation with humates can influence milk composition. Although the mechanism of HS administration in milk synthesis has not been fully described, their beneficial effects on gastrointestinal processes and nutrient utilization can probably increase the crude protein, pure protein and casein content in milk. Higher protein and especially casein content in milk could be very important for the cheesemaking in context of the cheese yield.

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