Agronomy Research **21**(3), 1246–1253, 2023
https://doi.org/10.15159/AR.23.054

Effect of new biologically active feed ingredient - potassium humate on productivity and milk quality of dairy cows

A. Nolberga-Trupa[1,*], D. Ruska[2] and G. Grandbergs[3]

[1,2]Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Institute of Animal Sciences, 2 Liela Street, LV-3001 Jelgava, Latvia
[3]Peasant facility 'Dukati', Vitinu Parish, LV-3708 Auces Municipality, Latvia
[*]Correspondence: aiga.trupa@lbtu.lv

Received: January 31st, 2023; Accepted: June 19th, 2023; Published: June 27th, 2023

Abstract. In animal feeding, the use of effective bioactive feed additives of natural origin, which have a stimulatory effect on the digestive and metabolic processes in an animal organism, while maximally maintaining the conditions for a healthy rumen environment, in combination with an environmentally friendly and economically beneficial agricultural production process, is being studied. Humates can play an important role in addressing this problem. Therefore, a study with Holstein-Friesian Black-and-White cows was carried out on the farm 'Dukati', Vitinu Parish, Auce Municipality, to understand the effect of potassium humate on the productivity and milk quality of dairy cows. Animals were selected for the research and assigned by the analogy principle to two treatment groups: trial and control; each group consisted of 15 dairy cows. The dairy cows were kept in free stall barns. The animals were provided with all welfare requirements according to the guidelines. During the trial, a decrease in productivity in both groups of cows was observed, but this decrease was slower in the trial group - by 6 kg ECM, compared to the beginning of the trial. During the trial, the milk composition, according to its fat and lactose content, was similar for cows of both groups. The protein content in the milk of the cows of both groups differed ($P < 0.05$). In the trial cow group, the protein content in milk increased by 0.15%, but the urea content in the milk decreased by 0.96 mg dL^{-1}, and somatic cell count by 180 thsd mL^{-1} compared to the beginning of the trial.

Key words: dairy cows, potassium humate, productivity, milk quality.

INTRODUCTION

The genetic potential, productivity and milk quality of animals can be realised through balanced diets, which is why the use of effective bioactive feed additives of natural origin is being explored in animal feeding. Animal diets lacking in protein, energy and other main nutrients result in a deficiency of biologically active principles, which causes metabolic disorders and can significantly affect the health and productivity of the animal. The problem has become urgent owing to incorporating various, including synthetic, feed supplements into animal diets. Therefore, possibilities are sought to develop effective biologically active feed supplements of natural origin, while maximally maintaining the conditions for a healthy rumen environment. One source of bioactive substances is humates. Humates are formed from chemical and biological decomposition of plant, animal and microbial materials mostly by soil bacteria. Today, humates have a wide application in various fields of human activity. They are used in construction and medicine, as well as in agriculture - crop and livestock production. The applications of humic substances in animal husbandry are diverse, but their use as feed additives is not sufficiently developed.

Researchers in different countries have demonstrated that the humic substances in the animal body operated on the cellular and subcellular level, as they did in plants. They enter the cell and are involved in metabolic processes, contributing to the optimisation of the passage of inorganic ions through the intestinal wall, thereby promoting the absorption of minerals, necessary for the normal functioning of the organism. This is how the stimulating influence of humic substances on separate systems and on the organism is manifested, as humic substances from different natural materials were tested in different branches of animal husbandry (cattle, pig, poultry, etc.) and all the information obtained present convincing evidence of the high efficiency of humates (Kucukersan et al., 2005; Wang et al., 2008; McMurphy et al., 2011; Degirmencioglu, 2014; Bezuglova & Zinchenko, 2016). Scientific research studies have proven that humic substances contribute to the absorption of nutrients in animals, increase their milk yield by 15–20%, the quality of milk and reproductive performance and the live weight of new-born calves by 22.4% as well as strengthen the immune system (Trckova et al., 2005; Kouřimská et al., 2014; Potůčková & Kouřimská, 2017; Tomassen & Faust, 2018; Yüca & Gul, 2021). The increasing demand for feed supplements of natural origin by livestock farmers aroused interest in starting research on raw materials derived from natural resources available in Latvia. The aim of the study was to investigate the effect of potassium humate, a new biologically active feed ingredient from Latvian black peat, on the productivity and milk quality of dairy cows.

MATERIALS AND METHODS

Research place, time, animal keeping and feeding

Trials were carried out on the peasant facility 'Dukati', Vitinu Parish, Auce Municipality. For the research, Holstein-Friesian Black-and-White cows were selected and assigned by the analogy principle (according to yield, lactation, lactation phase and live weight) to two treatment groups: trial and control, with 15 dairy cows in each group. The average live weight of cows in both groups was 700 kg, the mean age was 2.0 lactations. Cows with an average yield of 40.29 kg per day, fat content of 3.47% and protein content of 3.44%, lactation days 121–250 were included in the research. The trial was carried out from 1 October 2020 to 31 December 2021. Before starting the dietary trial there was an adaptation period for the cows from 15 to 30 September 2020, i.e., for 15 days. The research design is presented in Table 1.

Table 1. Scheme of the trial

Group of cows	Number of cows	Basic feed ration
Trial	15	Partial mixed ration (PMR) + 7 g potassium humate per cow per day
Control	15	Partial mixed ration (PMR)

The dairy cows were kept in the same rearing and feeding conditions. The dairy cows were kept in free stall barns. The cows had a deep bedding area in the barn, where straw was used for bedding. Bedding was replenished once a week. Animals were allowed *ad libitum* access to water and fed six times a day (at 5:00, 8:00, 13:00, 17:00, 20:00 and 22:30) with PMR (Table 2 and Fig. 1) prepared according to the Nutrient Requirements of Dairy Cattle (NRC, 2001). The animals were provided with all welfare requirements according to the guidelines. During the study, no animal diseases were detected.

Table 2. Chemical composition of PMR

Component	Content
Dry matter, %	40.7
Crude protein (CP), % DM	15.4
Degradable intake protein (DIP), % CP	48.0
Undegradable intake protein (UIP), % CP	32.9
Crude fibre, % DM	17.2
Net energy for lactation (NEL), MJ kg^{-1} DM	7.1
Crude fat, % DM	4.1
Neutral detergent fibre (NDF), % DM	32.4
Acid detergent fibre (ADF), % DM	19.8
Calcium (Ca), % DM	0.91
Phosphorus (P),	0.44

Figure 1. Feeding robot - Trioliet.

The PMR consisted of, calculating per cow per day: 20 kg silage maize (37.03% by mass), 27 kg lucerne silage (50% by mass), 1 kg lucerne hay (1.86% by mass), 3 kg (5.56% by mass) of grain mixture (wheat, barley) from the company 'Baltic Agro', 1.70 kg rapeseed meal (3.15% by mass), 0.50 kg Probut syrup (0.93% by mass), 0.20 kg UFAC Omega 3 fat additive (0.37% by mass), 0.05 kg sodium chloride (0.09% by mass) 0.25 kg Vetimplex-Likra-Gold mineral additive (0.46% by mass), 0.20 kg Osteovet mineral additive (0.37% by mass) and 0.1 kg Provital Pro lac mineral additive (0.18% by mass). The difference between the feeding of one group and the other was that the cows in the experimental group received potassium humate 7 g per cow per day which mixed with supplementary feed (pelleted) consisting of maize, barley, soybean meal and molasses (liquid) from the company 'Baltic Agro', which the cows received in the milking robot depending on the level of productivity (Fig. 2).

The feeding ration varied according to each cow's milk yield and physiological state, and was corrected monthly depending on the results of the control milk yield, dry period and state of health.

Figure 2. Feed component potassium humate.

Cows were milked on average 3.1 times a day in the milking robot. Each cow's individual milk yield and whole group average content of milk fat and protein was recorded, based on the control milk yield sheets. The control of the milk yield in cow groups was performed each day, individual milk yields were controlled once a month.

Chemical analysis methods and statistical analyses

Full value nutrition analysis was conducted by the accredited Biotechnology scientific laboratory of Latvia University of Life Sciences and Technologies following the accredited ISO standard methods and 'J.S Hamilton Baltic' according to the generally accepted methodology. Chemical analysis of the feed samples was carried out in accordance with the ISO 6498:1998 standards, dry matter - Feed Analyses met. 2.2.1.1:1993, crude protein, DIP, UIP-LVS EN ISO 5983–2: 2009, crude fat - ISO 6492:1999, crude fibre - ISO 5498: 1981, neutral detergent fibre (NDF%) - LVS EN ISO 16472:2006, acid detergent fibre (ADF%), net energy for lactation (NEL, MJ kg^{-1}) - LVS EN ISO 13906:2008, calcium - LVS EN ISO 6869:2002 and phosphorus - ISO 6491:1998. The content of phosphorus was determined using the spectrophotometry method, the content of calcium, magnesium, sodium, potassium, etc. minerals was determined using the atomic absorption method. Amino acid tests were performed by means of AccQ Tag technology (Waters Corp., Miliford, MA) and quantified by means of Shimadzu HPLC (low pressure gradient system). Beta-carotene by EN 12823-2:2000 / LC-DAD, vitamin A by EN 12823-1 2014, vitamin C by ISO 20635:2018/LC-DAD, vitamin D_3 by EN 12821:2009/LC-DAD, vitamin E by EN 12822:2000, vitamin B_1 by EN 14122-2014 / LC-FLD, vitamin B_2 by EN 14152:2006 mod. / rp - HPLC-FLD and vitamin B_{12} by J. AOAC 2008, vol 91 No 4 / LC-UV/DAD. Humic and fulvic acids tests were performed by LVP D-21:2018, 1 edition prepared according pagal Method Agricultural Chemical Analysis. Method 5.4. Cabi Publishing, 2002.Fat, protein, lactose, urea content in the milk was analysed using the instrumental infrared spectroscopy method according to ISO 9622 / IDF 141:2013 using MilcoScan (Foss, Denmark). The number of somatic cells was analysed using the instrumental flow cytometry method LVS EN ISO 13366-2:2007, using Fossomatic TM FC (Foss, Denmark) in an accredited milk quality laboratory SIA 'Piensaimnieku laboratorija'. In order to compare and evaluate the results between the groups, the amount of energy-corrected milk (ECM) per kg per day^{-1} was calculated using the following formula (Garcia et al., 2006):

$$ECM = \text{Milk yield x } \frac{0.383 \text{ x Milk Fat, \%} + 0.242 \text{ x Milk Protein, \%} + 0.7832}{3.14} \quad (1)$$

where *ECM* – energy-corrected milk.

The biometric data was processed using the computer program MS Excel. The differences between the group averages were determined by the *t–test* at the level of significance $\alpha = 0.05$.

RESULTS AND DISCUSSION

Chemical composition of potassium humate

The chemical composition of potassium humate is shown in Tables 3 and 4. As can be seen from Tables 2 and 3, the chemical composition of potassium humate (dark brown natural organic powder) contains a wide variety of biologically active components - minerals, amino acids, vitamins and other biologically active substances that can significantly increase the biologically functional value of the feed ingredient. Humic acid is an important component of the bioactive substances in potassium humate. Due to its specific chemical composition, humic acid reacts with biologically active substances such as choline, thiamine, riboflavin, nicotinamide, pantothenic acid and is able to absorb various harmful substances - toxic heavy metals, pesticides, radionuclides, etc., ensuring their elimination from the body, thus acting as a bio-regulator.

The use of biologically active substances, including humic substances, activates digestive and metabolic processes in the animal organism, promoting the transformation of feed nutrients in available forms, raising daily milk production and milk quality (Bezuglova & Zinchenko, 2016; Kucukersan et al., 2005).

A total of 16 amino acids were detected in the potassium humate. The highest concentrations of amino acids present in the potassium humate were aspartic acid, glutamic acid, proline, alanine and glycine.

The serine, threonine and valine of the feed ingredient was similar. The lowest concentrations of amino acids present in the potassium humate were methionine, cystine and arginine.

Table 3. Chemical composition of potassium humate

Determined component and its unit of measurement	Test result
Dry matter, %	94.66
Crude protein, %	3.76
Crude fibre, %	38.87
NEL, MJ kg^{-1}	3.03
Crude ash, %	31.08
Calcium, %	1.68
Magnesium, %	0.22
Potassium, %	5.92
Sodium, %	0.11
Zinc, mg kg^{-1}	7.67
Copper, mg kg^{-1}	2.00
Manganese, mg kg^{-1}	59.45
Iron, mg kg^{-1}	2,970.89
Beta-carotene, μg 100 g^{-1}	< 5
Vitamin A, μg 100 g^{-1}	< 21
Vitamin D3, μg 100 g^{-1}	< 0.25
Vitamin E, mg 100 g^{-1}	5.79
Vitamin C, mg 100 g^{-1}	< 0.5
Vitamin B1, mg 100 g^{-1}	< 0.015
Vitamin B2, mg 100 g^{-1}	< 0.01
Vitamin B9, μ 100 g^{-1}	< 5
Vitamin B12<, μg 100 g^{-1}	< 0.25
Humic acid, %	4.17
Fulvic acid, %	2.0

Table 4. Amino acid content of potassium humate, on dry matter basis, g 100 g^{-1}

Amino acids	Test result
Alanine	0.10
Arginine	0.04
Aspartic acid	0.13
Cystine	0.02
Phenylalanine	0.07
Glycine	0.10
Glutamic acid	0.13
Histidine	0.04
Isoleucine	0.06
Leucine	0.10
Lysine	0.05
Methionine	0.01
Proline	0.12
Serine	0.08
Threonine	0.09
Valine	0.09

Productivity and milk quality during the trial

Productivity is one of the most important indicators of the effect of one or another dietary factor on the cow's production performance (Islam et al., 2005; Batchelder, 2000). Cow productivity during the trial is shown in Table 5. As we can see, milk yields decreased in both cow groups during the research, while statistically significant differences between the average daily milk yield were not observed in either group (P < 0.05). A more rapid decrease in milk yield was observed in the control group - by 6.4 kg per day; this decrease was slower in the trial group - by 6 kg ECM, compared to the beginning of trial. However, these changes in productivity are more related to the physiological processes during the lactation and gestation cycles of cows.

Table 5. Cows' productivity during the trial, on average, kg

Group of cows	Beginning of trial	Middle of trial	End of trial
Trial	40.7 ± 7.39	37.3 ± 16.28	34.7 ± 8.37
Control	40.5 ± 7.94	33.8 ± 13.54	34.1 ± 13.54

Table 6. Changes in milk composition during the trial

Group of cows	Milk components	Beginning of trial	Middle of trial	End of trial	Compared to the beginning, ±
Trial	Fats, %	3.47 ± 0.52	3.83 ± 1.02	3.84 ± 0.63	+0.37
	Protein, %	3.44 ± 0.24	3.25 ± 0.31	3.59 ± 0.23	+0.15
	Lactose, %	4.87 ± 0.19	4.78 ± 0.22	4.77 ± 0.12	-0.10
	Urea, mg dL^{-1}	28.17 ± 4.23	19.55 ± 7.93	27.21 ± 3.34	-0.96
	SCC, thsd mL^{-1}	334 ± 123	512 ± 110	164 ± 153	-180
Control	Fats, %	3.65 ± 1.05	3.81 ± 1.02	4.04 ± 0.82	+0.39
	Protein, %	3.50 ± 0.34	3.36 ± 0.40	3.38 ± 0.40	-0.12
	Lactose, %	4.90 ± 0.16	4.84 ± 0.22	4.80 ± 0.19	-0.10
	Urea, mg dL^{-1}	26.35 ± 2.69	20.14 ± 7.21	29.32 ± 3.83	+2.97
	SCC, thsd mL^{-1}	116 ± 112	220 ± 143	876 ± 131	+760

Changes in milk composition during the trial are shown in Table 6. During the trial and lactation period, alongside the decrease in productivity of the cows, it was possible to observe an increase in fat content in the milk for the cows of both groups, respectively by 0.37% and 0.39% compared to the beginning of the trial. The content of fat in the milk decreased by 0.20% in the trial group compared to the control group. A greater difference could be observed concerning the protein content in the milk of the trial cow group, which was higher by 0.15%, but in the control group the protein content decreased by 0.12% compared to the beginning of the trial (P < 0.05). The content of protein in the milk increased, on average, by 0.21% in the trial group compared to the control group. The protein content of milk mainly depends on the hereditary characteristics of the animal; however, it is also affected by the protein content of feed and the absorption of the protein, which are related to protein metabolism (Xiaowang et al., 2010; Yüca & Gul, 2021). The content of lactose only changed slightly in the milk; it has an insignificant tendency to decrease by 0.10% for the cows of both groups, compared to the beginning of the trial (P < 0.05). The content of lactose in the milk decreased by 0.03% in the trial group compared to the control group. Lactose content in milk is closely dependent on the health status of each cow and decreases with increasing lactation.

Composition of feeding ration and nutrition has less influence on the lactose content (Potůčková & Kouřimská, 2017). During the research, potassium humate had a favourable influence on the decrease in content of urea and somatic cell count in the milk. The content of urea in the trial group decreased, on average by 2.11 mg dL^{-1} compared to the control group, and by 0.96 mg dL^{-1} compared to the beginning of the trial but this could be explained by the stimulatory effect of potassium humate on the change of animal proteins in the body. The variability of the urea level in milk is due to many factors - age of the cows, lactation period, milk yield, season of the year, connection with other indicators of milk composition ($P < 0.05$).

As we can see, the somatic cell count in milk for the cows of the trial group decreased compared to the control group and compared to the beginning of the trial ($P > 0.05$). Milk Somatic Cell Count is a key measure of milk quality, reflecting the health status of the mammary gland and the risk of non-physiological changes to milk composition. The somatic cell count in milk is also influenced by other factors: the type and time of milking, the period of lactation, the season, the animal's productivity, age and breed and genetic predisposition (Xiaowang et al., 2010; Tomassen & Faust, 2018).

CONCLUSIONS

In the research, a decrease in productivity was observed in both groups of cows, but a more rapid decrease in milk yield was observed in the control group. The milk composition, according to its fat and lactose content, was similar for cows of both groups. The fat content in milk had a tendency to increase alongside the decrease in milk yield during lactation, except for the content of lactose that only slightly decreased in the milk. The results of the study confirmed the stimulatory effect of potassium humate on metabolic processes in the animal organism and contributed to a 0.15% increase in protein content in milk, a 0.96 mg dL^{-1} decrease in urea content and a decrease in somatic cell count in milk compared to the beginning of the study. However, in each case, more research is needed, varying the dosage and the schedule of their use.

ACKNOWLEDGEMENTS. The research was carried out with the financial support of project No. 19-00-A01620-000076 'Testing of new feed ingredients from Latvian black peat - potassium humate - included into diets for dairy cows' funded by the Ministry of Agriculture and the Rural Support Service and implemented under the EU EAFRD and the Rural Development Programme of Latvia for 2014–2020, measure 16 Cooperation, sub-measure 16.2 Support for the development of new products, methods, processes and technologies.

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