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EFFECTS OF DIETARY BETAININE ON PRODUCTIVE TRAITS AND REPRODUCTIVE HEALTH OF DAIRY COWS

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Summary. Dietary supplementation of betaine may play an important role in productive and reproductive parameters of dairy cows. The aim of this study was to define the role of dietary betaine in dairy cows' lactation. Cows were assigned to betaine or control groups according to the 'case-control' study design. Statistical methods included Pearson's chi-squared and *t* criteria, Pearson and Spearman correlation coefficient *r* and ANOVA.

We observed positive results after betaine diet correction of milk and reproductive traits of dairy cows in the heat stress conditions. Homocysteine level in blood of dairy cows is not depend on the age of the animals ($r = 0.09$), on the amount of lactation ($r = 0.04$), on a period after calving ($r = -0.07$). In this period higher milk yield was observed on lower homocysteine levels in the blood plasma of animals ($r = -0.32$). Also we found out that more inseminations were required for animals with a higher homocysteine level in the plasma ($r = 0.36$).

After betaine supplementation milk fat concentration was higher in betaine-treated group of cows compared with control cows (3.05 vs 2.74%). Milk yield in betaine-treated group was in the negative correlation with milk fat ($r = -0.67$). An analysis of milk yields dynamics had showed that cows fed betaine had more stable and predictable milk yields per milking than controls (8.3 vs 40%) and milk yield in BET group was in a negative correlation with milk fat ($r = -0.67$). Negative dynamic of homocysteine level was noticed in a betaine group (27.5%) and positive (19.4%) — in the control group. More inseminations were required for cows of control group (1.8 vs 1.2).

Keywords: dairy cows, one-carbon metabolism, homocysteine, betaine, milk traits, reproduction

Introduction. One-carbon metabolism of mammals is one of the key points of metabolism and its research is a perspective direction for the development of pharmacological correction of failures. The actual figure is to assess the biochemical metabolism of dairy cows as a basis for further selection and analysis of genes, causing the reproductive traits of animals.

An adequate folate and vitamin-B status is critical to maintain the optimal efficiency of the homocysteine-methionine cycle. It is known that SNPs of one-carbon metabolism human genes are associated with — carcinogenesis (colorectal adenocarcinoma, breast cancer and ovarian cancer); cardiovascular disease (ischemic heart disease — coronary heart disease, myocardial infarction, atherosclerosis, atherothrombosis); pregnancy complications (placental insufficiency, premature detachment of normally situated placenta, late gestosis); fetal malformation (cleft neural tube, anencephaly, deformities of the facial skeleton), children born with a chromosomal abnormality, etc., because its synthesized enzymes play an important role in folate metabolism, which is an integral process for DNA and RNA synthesis

and in protein methylation. So this MTHFR enzymatic activity process is lowered in subjects with MTHFR 677TT and 677CT genotypes and these individuals might require an increased intake of folate or another dietary factors to maintain or control blood levels of plasma folate or homocysteine (Maroto-Sánchez et al., 2016; Asim et al., 2015; Vanilla et al., 2015; Bertoia et al., 2014; Rai, 2011; Ciaccio, Bivona and Bellia, 2008; Forges et al., 2007; Altomare, Adler and Aledort, 2007; Doolin et al., 2002; Zetterberg et al., 2002).

Homocysteine is an intermediate sulfur-containing amino acid involved in the methionine cycle and could be converted to methionine due to the remethylation reaction requires BHMT and both folate and vitamin B₁₂ as coenzymes (Maroto-Sánchez et al., 2016; Eskandari et al., 2016; Xu et al., 2016; Jia et al., 2015).

Several studies of dairy cows had shown that plasma homocysteine levels are affected by diet factors such as protein and vitamin deficiencies by genetic background and by several pathological conditions. Moreover, the homocysteine in blood could be also associated with chronic inflammatory bowel disease (Crohn's disease and

the variations of vitamin B₁₂ and subacute ruminal acidosis). It was found an increase of vitamin B₁₂ values in dairy cows with lower rumen pH and a decrease of homocysteine values in dairy herds with lower rumen pH (Cannizzo et al., 2012; Roblin et al., 2006).

It has been reported by Başbuğan, Yüksek and Altuğ (2015) about analysis of the diagnostic and prognostic significance of homocysteine and cardiac troponin I and routine cardiac parameters, in cows with hypocalcemia, because hypocalcemia causes a decrease in myocardial contractions, left ventricle systolic dysfunction, and thus systolic heart failure, and an increasing homocysteine levels in blood could be a signal for coronary heart disease, paralysis, peripheral vascular disease, and intravascular thrombosis.

Many articles have documented that correction and maintenance of one carbon metabolism level could be carried out with a dietary factors for human and animals, such as betaine (Saeed et al., 2017; Di Pierro, Orsi and Settembre, 2015; Jia et al., 2015; Maclean et al., 2012).

Betaine or tri-methyl glycine, is a natural compound either produced endogenously by choline oxidation or found in feed ingredients, such as sugar beet solubles and a key component in one-carbon metabolism. Betaine has two main functions in an animal's body. It is a powerful osmolyte to reduce dehydration and stabilize protein when a cell is under stress condition and it serves as a methyl donor when fed to animals (Monteiro et al., 2017; Tao et al., 2016; Zeisel, 2013; Bertolo and McBreaity, 2013; Eklund et al., 2005). Betaine reduces the lipid components in the liver and prevents fatty liver disease. The lipotropic effect is associated with the transfer of a methyl group from homocysteine to S-adenosylmethionine through methionin. In this reaction, betaine, choline and methionine supply the methyl group (Nakai et al., 2013).

It was presented by Hall et al. (2014) that betaine is a molecular chaperone and has been shown to decrease susceptibility of stress to microbial populations acts as an antimicrobial to some bacteria like *Salmonella typhimurium*, can be utilized as a nutrient and has been demonstrated to increase milk production.

Effects of betaine for animals metabolism in a heat stress conditions had been studied by different authors, because various studies indicate that parturition heat stress of dairy cows exaggerate the dysfunctional immune system during late gestation and early lactation. The humoral immune response is also altered by heat stress during the transition period (Tao et al., 2016). Late-gestation heat stress compromises placental development, which results in fetal hypoxia, malnutrition, and eventually fetal growth retardation (Tao and Dahl, 2013). According to Tao et al. (2016) and Thompson and Dahl

(2012) cows dried off in the hot months had increased incidences of mastitis, respiratory disorders and retained fetal membranes in early lactation compared with those dried in cool months.

Research of Hall (2014) had demonstrated that milk protein and lactose (%) increased with the middle dose of betaine during heat stress compared to the controls. Cows fed betaine had significantly higher milk production during thermal-neutral condition and elevated plasma glucose during heat stress. In this time nutritional supplementation of heat-stressed lactating cows has been widely studied, but the related research for dry cows under heat stress is somewhat limited, especially during the far-off period (Tao et al., 2016).

The same results were obtained after betaine supplementation for pigs and poultry (Saeed et al., 2017; Jia et al., 2015; He et al., 2015). Previous studies showed that dietary supplementation of betaine in poultry diets could positively affect nutrients' digestibility, reduce abdominal fat weight, and increase breast meat yield. Betaine may play an important role in lean meat production by positively affecting the lipid metabolism with increased fatty acids catabolism and thus reducing carcass fat deposition (Saeed et al., 2017). In the paper by He et al. (2015) about broiler betaine diet the control and betaine-supplemented broiler groups it was described higher feed consumption, body weight gain, and lower feed: gain ratio compared with the heat stress S-control group. Betaine supplementation significantly decreased triglyceride, free fatty acids, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol. Authors had shown that chronic heat stress reduces broiler production performance. However, betaine can reverse these negative effects partially and thus improve carcass composition by changing lipid metabolism.

At the same time it could be noted that the ambiguity and insufficiency of data on the effect of betaine on the productivity and reproduction of dairy cows. There are a number of factors that affect one-carbon metabolism such as age, gender, nutrition, genetics, medication, physical activity, climatic conditions and animals' husbandry conditions. That is why it is beneficial to conduct research on the Ukrainian selection breeds in the technological and fodder conditions of Ukrainian farms.

The aim of the study was to evaluate the role of dietary betaine in lactating dairy cows.

Materials and methods. A study was conducted at the State Enterprise Research Farm 'Nyva' of Institute of Animal Breeding and Genetics named after M. V. Zubets of the National Academy of Agrarian Sciences of Ukraine and V. N. Karazin Kharkiv National University during August–early September 2016.

The type of production in SE RF 'Nyva' is organic. Cows had milk production (6,514 l), milk fat (3.65%) and milk protein (3.20%) concentration during 2016 in this farm. The base total mixed ration (TMR) was alfalfa haylage based with corn silage, corn and other silage. The system of keeping cows is traditional. Part of time animals spent in the walking areas.

To study the potential impact of supplementation of betaine containing diet, 35 cows of Ukrainian red-spotted milk and Ukrainian black-spotted milk breeds at the age of 1st–7th lactation after calving were randomly assigned to betaine or control groups according 'case-control' study design.

All animals had the same light/dark schedule, humidity and temperature. The air temperature at this time of year in the daytime was 22–34 °C outside, and 18–26 °C — in the cows' room.

Cows were fed individually. Betaine-hydrochloride, Beta-Key ('Orffa', Netherlands) was used as the source of studied supplement. The control group received 0 g/d, the betaine dose was fed at 40 g/d once a day. The supplement was mixed with grain mixture. A few days we spent to adapt betaine group to the new diet.

Cows were milked from 6 to 8 a.m., and from 5 to 7 p.m. daily. Samples were individually stored and analyzed by Ecomilk-Standart ('Bulteh 2000' Ltd., Bulgaria) for fat and protein contentment. The plasma homocysteine levels were analyzed using commercial ECLIA test kits. Production, reproduction and biochemical parameters were analyzed before and after betaine supplementation and compared against control to understand the effects of betaine.

Statistical analysis was performed with the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality and hypotheses — criteria *t* and χ^2 . The relationship between traits was estimated by the Spearman and Pearson correlation analysis. Means for two groups were compared by ANOVA (Atramentova and Utevskaia, 2008).

Results and discussion. The analysis of the results obtained in the present study indicated that before experiment betaine-treated cows group (BET) and control group (CONT) had the same milk yield, milk fat and protein contents, number of inseminations for successful result and homocysteine level was little higher in one of the groups ($p = 0.044$) (Table 1).

Table 1 — Characteristics of CONT and BET groups

Parameters of the cows	Results of study			
	Before study		After study	
	CONT	BET	CONT	BET
Milk yield, l	21.20 ± 1.30	19.31 ± 1.42	16.10 ± 1.10	17.23 ± 0.92
Milk fat, %	3.60 ± 0.01	3.42 ± 0.08	2.74 ± 0.17	3.05 ± 0.19
Milk protein, %	2.86 ± 0.01	2.75 ± 0.9	3.07 ± 0.02	3.1 ± 0.02
Homocysteine level, Mmol/l	4.31 ± 0.26	7.91 ± 1.81	5.15 ± 0.39	5.73 ± 0.62
N	1.6 ± 0.1	1.41 ± 0.1	1.8 ± 0.1	1.2 ± 0.1

Notes: CONT — control group of cows, BET — betaine group of cows, N — number of the last successful insemination.

At the end of experiment we found statistically significant decreasing of milk fat — by 23.9% ($p = 0.02$) in CONT and by 10.8% ($p = 0.01$) in BET, milk yield — by 18.8% ($p = 0.009$) in CONT only, but in BET group this trait was without differences ($p = 0.17$). Milk protein levels increased — by 7.3% ($p = 0.004$) in CONT and by 12.7% ($p = 0.02$) in a BET group (Table 1).

Such dynamics of milk traits is typical for most Ukrainian farms under conditions of heat stress and increasing of green mass in feed during summer period.

It is important to note that we observed the statistically significant higher milk fat concentration (3.05 vs 2.74%, $p = 0.000001$) in the BET cows group compared to the controls one after betaine supplementation. Milk yield and milk protein were the same in the both groups. Milk yield in BET group showed negative correlation to

milk fat — $r = -0.67$ ($p = 0.037$) and we found no association between these traits in control.

In accordance with the data of Tao et al. (2016) cows that received betaine diets starting at 56 d before the expected calving date had improved not only milk fat concentration (4.78 vs 4.34%), but milk production (44.2 vs 41.5 kg/d), compared with control cows. The results of Zhang et al. (2014) showed that feeding betaine to cows increased feed intake, milk yield, milk lactose, milk protein, plasma cortisol, glutathione peroxidase, superoxide dismutase, and malondialdehyde levels ($P < 0.05$). In contrast, it has been reported by Hall (2014) that dietary betaine increased milk yield and percent protein during the thermoneutral period. In 2016 Hall et al. conclude that dietary betaine increased milk yield during the thermoneutral conditions, but no differences

were found between betaine and control in total milk production or milk composition during heat stress, that partially consistent with our results. The research of Peterson et al. (2012) had demonstrated that supplementing 100 g/d rumen-unprotected betaine increased milk production in mid-lactation cows under a thermal-neutral condition. Monteiro et al. (2017) reports that cows enrolled at dry off, with betaine support demonstrated higher milk yield (45.1 vs 41.9 kg/d) and fat content (4.78 vs 4.34%) and elevated plasma concentrations of non-esterified fatty acids and β -hydroxybutyrate in early lactation compared to control.

The dynamics of milk yields that we observed at the end of the intake of betaine from BET cows was noted. It was from 0 to 3 liters per milking, 1.6 liters on average, in the control group cows — 0–9 liters, on average 3.3 liters. Cows fed betaine had more stable and predictable milk yields per milking than controls (8.3 vs 40%, $p < 0.05$).

It is well known that different dietary components as methionine content, riboflavin, alcohol or coffee consumption are being investigated in relation to homocysteine concentration (Varela-Moreiras, 2001). That is why the actual figure is to assess the

homocysteine level in blood of dairy cows as trait of one-carbon metabolism and target for correction by betaine.

The analysis showed that the homocysteine levels in blood of animals before experiment was in the range from 2.96 to 27.9 mmol/l, reaching an average of $5.72 \pm 0.73 \mu\text{mol/l}$. Homocysteine level in blood of dairy cows (Table 2), not dependent on the age of the animals ($r = 0.09$, $p = 0.57$) or, respectively, the number of lactation ($r = 0.04$, $p = 0.81$). This is comparable to human characteristics — the level of homocysteine increases during puberty and in adults its parameters accordance to the genotype, although in adulthood it may gradually increase (Maroto-Sánchez et al., 2016; Guttormsen et al., 1996).

No association between homocysteine level in the blood of animals and a period after calving ($r = -0.07$, $p = 0.68$) was found. It is known that during normal pregnancy, the level of homocysteine in humans tends to decrease, which usually occurs at the border of the first and second trimester of pregnancy, which promotes placental circulation, but after 2–4 days postpartum, the level of homocysteine is restored (Maroto-Sánchez et al., 2016; Guttormsen et al., 1996).

Table 2 — Relationship between the homocysteine level and the signs of cow

Parameters of the cows	Parameters of study before betaine feeding		
	Homocysteine level, Mmol/l	r	p
Age of an animals, 3–9 years	2.96–27.79	0.09	0.57
Last lactation number, 1–7	2.96–27.79	0.04	0.81
The time after calving, 2–68 days	2.96–27.79	-0.07	0.68
Number of the last successful insemination, 1–4	2.96–27.79	0.36	0.05
Milk yield, 11–23 kg/d	2.96–27.79	-0.32	0.07

Notes: r — correlation coefficient, p — significance level.

Before the study of betaine diet we found a statistically significant direct correlation between homocysteine level in the blood and the number of inseminations which were carried out before successful fertilization and development of pregnancy of cows — $r = 0.36$ ($p = 0.05$). More insemination was required for animals with a higher level of amino acid in the blood plasma (Table 2).

The literature data have demonstrated that in women the lower level of homocysteine in the follicular fluid is associated with a better chance of clinical pregnancy (Ocal et al., 2012). In addition, in patients with polycystic ovary syndrome, when comparing glucose and estradiol with healthy women, the level of homocysteine in the follicular fluid was higher with a significance level of $p = 0.01$ (Eskandari et al., 2016). It was also noted that homocysteine concentration in follicular fluid in women with polycystic ovary syndrome is associated with lower quality and number of oocytes and embryos (Berker et al., 2009).

The value of milk yield of cows showed a negative correlation with homocysteine level — $r = -0.32$, at a significance level of $p = 0.07$. Higher yield observed at lower homocysteine levels in the blood plasma of animals. It is known that due to functional or genetically determined defects of enzymes, in vitamin deficient conditions, deficiency of vitamins B₁₂, B₆, B₁, folic acid, the level of homocysteine in the cells increases and enters to the extracellular space and blood plasma. Cytotoxic effect of elevated homocysteine levels is dangerous especially for endothelial cells that affect the processes of reproduction and synthesis of milk (Maroto-Sánchez et al., 2016; Ciaccio, Bivona and Bellia, 2008; Guttormsen et al., 1996).

After experiment in the BET group the level of homocysteine decreased by 27.5%, in the CONT group it increased by 19.4% and equaled in a both groups (Table 1). The number of inseminations required for successful fertilization and development of pregnancy in

the CONT group increased by 12.5%. At the same time, this trait decreased by 14.3% in the group of BET cows. More insemination was required for cows of CONT group, 1.8 vs 1.2 ($p = 0.035$).

We hypothesized that that decreasing of homocysteine level and its cytotoxic effect contributed to successful inseminations and healthy pregnancy. Besides that participation of betaine in the conversion of homocysteine to methionine probably improved fertility of cows.

It is known that betaine, by virtue of aiding in the remethylation of homocysteine, removes both toxic metabolites (homocysteine and S-adenosylhomocysteine), restores S-adenosylmethionine level, reverses steatosis, prevents apoptosis and reduces both damaged protein accumulation and oxidative stress (Kharbanda, 2009). With participation of betaine homocysteine could be converted to methionine. This is an important in a case when methionine is one of the most limiting amino acids in dairy cows (Vailati-Riboni et al., 2017).

Different works had established that cows around calving time experience a depression on immune function partially due to the marked negative energy balance, which results when cows cannot ingest enough nutrients to support dietary requirements for milk production. It has been reported that methionine plays a key role in milk protein synthesis, hepatic lipid metabolism, and immune function (Li et al., 2016; Osorio et al., 2013; Soder and Holden, 1999; Chen et al., 2007). Besides its crucial role in milk production, methionine and its derivate metabolites (e.g., glutathione, taurine, polyamines) are well-known immunonutrients in nonruminants (Vailati-Riboni et al., 2017) and well-established sources of the an oxidants (Li et al., 2016; Kim, Weiss and Levine, 2014; Luo and Levine, 2008; Moskovitz et al., 2001; Stadtman et al., 2002). So, rumen-protected methionine has been reported to increase lactation performance, health status and reproductive function of dairy cows (Hansen, 2016; Ardalan, Rezayazdi and Dehghan-Banadaky, 2010).

A role of key components of one carbon metabolism — homocysteine, methionine, betaine — in a reproduction of mammals had presented by authors from different countries. The critical relationship between perturbations in the mother's homocysteine and methionine metabolism and its impact on fetal growth and development is becoming evident (Kalhan and Marczewski, 2012). Effect of maternal methionine supplementation on the transcriptome of bovine preimplantation embryos had been described (Peñagaricano et al., 2013).

Thus, methionine has an essential role in the development of the bovine embryo from morula to blastocyst (Ikeda, Sugimoto, and Kume, 2012). Research of Luchini (2014) had demonstrated that supplementation of cows with methionine resulted in lower pregnancy losses from cows fed methionine-enriched diets, suggesting that methionine favors embryo survival, at least in multiparous cows.

Conclusions. We observed positive results after betaine diet correction of milk and reproductive traits of dairy cows in a heat stress conditions.

The relationship between productivity and reproduction traits of dairy cows — the value of milk yield and efficiency of insemination and the homocysteine level in blood of cows was shown.

After betaine supplementation milk fat concentration was higher in betaine-treated group cows compared with control cows (3.05 vs 2.74%). Milk yield in betaine-treated group was in a negative correlation with milk fat ($r = -0.67$).

Cows fed betaine had more stable and predictable milk yields per milking than controls (8.3 vs 40%).

Negative dynamics of homocysteine level was noted in a betaine group (27.5%) and positive (19.4%) in a control group. More insemination was required for cows of control group (1.8 vs 1.2).

Thus, the obtained results show the prospects for further pharmacogenetic research of dairy cows in Ukrainian farms.

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