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THE EFFECT OF DHA OMEGA-3 FEEDING IN THE HIGH YIELDING HOLSTEIN HERD

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ABSTRACT
The aim of this study was to analyse the effect of supplementary feeding of DHA (Docosahexaenoic Acid) rich algae product (Algae S^{TM} Alltech Inc.) on production of milk, fat and protein as well as on reproduction of high yielding Holstein dairy herd. Field trial was set up on Top 10 dairy farm in western part of Slovakia, under commercial conditions. The data of high yielding dairy cows, separated in two groups of 30 (control) and 29 (trial) animals, were recorded for period of 3 subsequent months from October to December 2015. Animals were fed once a day Total Mixed Ration based diet with different feed mixture composition in trial group (+100 g Algae S^{TM} Alltech Inc. per cow and day). Performance data were collected in accordance with official milk recording system of Breeding Services of Slovak Republic s. e. and milk samples were collected once per month according to the A4 standard methodology. The control group showed higher level of milk production compared to trial. Our study indicated that the feeding of algae caused milk fat depression and generally lower protein content in milk. Significant impact of algae feeding was found also for the level of urea in milk. In addition, the supplementary feeding of DHA may represent effective strategy to increase the percentage of pregnancies per inseminations in lactating dairy cows.

Keywords: cattle; algae; calving rate; milk production

INTRODUCTION
The dairy cow industry has changed dramatically over the past decades. Per-cow milk yields have increased markedly as a combined result of improvements in animal management, nutrition, and genetics. A prerequisite for good lactation performance during a cow's life span is the production of offspring at regular intervals. Good feeding strategies (composition, quantity, palatability, availability, and the access of the feed) are also important **(Leroy et al., 2013)**. **Nitrayová et al. (2014)** and **Frančáková et al. (2015)** studied fatty acids composition in different sources of plant seeds used for human nutrition and production of vegetable oil. Because corn and soybean are staple food crops for humans, their common use as the main source of dietary energy and protein for food-producing animals directly competes with their allocation for human consumption **(Lum et al., 2013)**. The metabolic demands for increasing milk production are significant and represent a major challenge to ensure optimum production while also facilitating reproduction. Dietary nutrients and nutrients from body tissues are directed to milk production. During this same time, the uterus, ovary, and hypothalamus/pituitary glands undergo a process of recovery and rebuilding for the establishment of subsequent pregnancy **(Thatcher et al., 2006, 2011)**.

Recent understandings of the role of fats in metabolism open new opportunities for improving production, health, and reproduction in cattle. Increasing evidence of positive effects of feeding fats during transition on fertility and the adaptation to lactation **(Rodney et al., 2015)** exists. Fertility could be enhanced by feeding n-3 diet due to the effect of n-3 PUFA on the concentration of prostaglandin F2 alpha (PGF2α) in the uterus, thus potentially facilitating embryo implantation and reducing embryo mortality **(Mattos et al., 2004)**. Diet enriched in n-3 PUFA increased the number of the pre-ovulatory follicles **(Ambrose et al., 2006)** and small follicles **too (Zachut et al., 2010)**. The essential fatty acids are the critical components of fats and one very important fatty acid is the omega-3 derivative, docosahexaenoic acid (DHA). The glucogenic diet was not successful in altering the milk fatty acid composition. On the other hand, direct rumen supplementation of 43.0 g of algae/kg of DM had dramatic effects on DMI and milk yield. Dietary supply of DHA-enriched microalgae resulted in an altered milk fatty acid composition. A modified milk fatty acid composition upon algae feeding was associated with decreased milk fat content when algae were supplemented with the diet. Good management during the transition period, in particular nutritional strategies, can reduce the effects of metabolic stress and improve production and reproduction **(De Veth**

et al., 2009). The decrease in fertility of the lactating dairy cow is multi-factorial and often associated with high milk production **(Lucy, 2001; Bernal-Santos et al., 2003)**. In recent years studies have examined the effects of feeding algal meal, high in DHA, on feed intake, enteric methane production and milk **parameters (Boeckaert et al., 2008; Stamey et al., 2012; Moate et al., 2013)**. Study of **Childs et al. (2008)** showed little difference in the effect of dietary n-3 or n-6 PUFA supplementation on range of reproductive parameters; however increased concentrations of PGFM have been found which could be beneficial for the formulation of post-calving diets of dairy cows. It has been demonstrated that feeding algal meal may inhibit voluntary dry matter intake and reduce milk fat concentrations **(Moate et al., 2013)**. Study of **Angulo et al. (2012)** observed that addition of algae for 10 weeks to the diet of dairy cows induced expression of lipogenic gene (SCD1, FASN) and reduction of the SREBF1 transcription factor gene expression in the mammary gland. Feeding rumen-protected marine algae to dairy cows enriched milk and butter with DHA **(Glover et al., 2012)**.

The aim of the study was to confirm the effect of supplementary feeding of DHA rich algae product (Algae STM Alltech Inc.) on production of milk, fat and protein as well as on reproduction of high yielding Holstein dairy herd.

MATERIAL AND METHODOLOGY

Field trial was set up on top 10 dairy farms in western part of Slovakia, under commercial conditions. It was performed in period of 3 subsequent months from October to December 2015. The analysed Holstein herd consisted of 400 cows, housed in free cubicles with straw bedding. Only first lactation high yielding dairy cows separated into the two groups of 30 (control) and 29 (trial) cows were included in study. Groups were consistent according the stage of production and reproduction cycle. Animals were fed once a day tmr based diet. The composition and nutritional parameters of fed tmr are shown in table 1 and 2. Both groups were fed using concentrate mixture with the same composition with only difference in algae/dha omega 3 feed supplement (algae s^{tm} alltech inc.), with dosage of 100 g per cow and day in trial group (Table 3).

The impact of algae feeding has been studied in relation to the milk production performance and calving rate as one of the most important reproduction parameters. Performance data were collected in accordance with official milk recording made on farm by Breeding Services of Slovak Republic s. e. Milking was performed three times a day. Performance recording and milk sample collection was made once per month according the of A4 standard methodology.

Statisic analysis

Collected data on daily milk production in kg, fat and protein content as well as urea were analysed using software SAS EG v 5.1 **(SAS Institute Inc., 2012)**. The significance of differences between groups of analysed parameters were tested by parametric statistic using Student two-sample t-test.

RESULTS AND DISCUSSION

Control and trial group were selected from first lactation cows to be consistent in stage of lactation (no significant difference between both groups in DIM), level of lactation (no significant difference in milk production, fat and protein content (Table 4, pre-trial)) and state of reproduction (postpartum cows). The significant difference (p >0.05) has been observed only in level of urea in milk -2.71mg.100 mL^{-1} in trial group (Table 1).

Slight higher (+1.34 kg) milk production in control group compared to the trial has been observed in October 2015. In contrary, the protein content was lower in control group (-0.13%). Observed differences were non-significant, while highly significant differences were observed in levels of fat (+0.98%) and urea (+10.71 mg.mL^{-1}) in favour of control group.

In November 2015 significant depression of milk production (-2.91 kg) was observed in trial group. Highly significant decrease of fat content (-0.67%, Table 2) and urea level (-7.60 mg.mL^{-1}) was found also in trial group. Protein content stayed almost untouched (diff. 0.02%).

Similarly, lower level of milk production was observed in December 2015, as well as the protein content (0.07%) in trial group, but the difference was non-significant.

Table 1 Composition of fed TMR.

Component	Control group	Trial group
Maize silage (%)	41.09	41.02
Alfalfa silage (%)	29.88	29.83
Feed mixture (%)	13.15	13.30
High moisture corn (%)	9.34	9.32
Gurmit (%)	5.60	5.59
Alfalfa hay (%)	0.56	0.56
Feed straw (%)	0.37	0.37

Table 2 Nutritional parameters of fed TMR.

Nutrient	Control group	Trial group
Dry matter (kg)	25.35	25.55
NEL (MJ)	160.90	160.26
Starch (%)	28.19	27.91
Fiber (%)	19.46	19.25
Crude protein (%)	16.80	16.57
Fats (%)	4.22	4.16

Table 3 Composition of supplementary feed concentrate mixture in both groups.

Component	Control group	Trial group
Algae (%)	-	1.40
Sunflower meal (%)	35.54	34.55
Barley seed (%)	28.43	27.64
Sunflower cake (%)	18.48	17.96
Megalac (%)	4.98	4.84
Rumagen (%)	4.98	4.84
Bicarbonate (%)	2.84	2.76
Vitamix (%)	2.13	2.07
Limestone (%)	1.42	2.76
Feed salt (%)	1.21	1.17

Table 4 Average production of milk and content of fat, protein and urea in milk during the trial in control (C) and trial (T) group.

Period	Group	Milk (kg ±SD)	Fat (% ±SD)	Protein (% ±SD)	Urea ($mg.100mL^{-1}$ ±SD)
Pre-Trial	**C**	27.56 ±5.04	3.10 ±0.63	3.04 ±0.26	27.77 ±5.79
	T	27.48 ±4.22	3.08 ±0.64	2.97 ±0.25	30.48 ±4.82
October 2015	**C**	31.92 ±6.83	3.75 ±0.73	3.27 ±0.28	27.26 ±7.82
	T	30.58 ±4.89	2.77 ±0.70	3.41 ±0.33	17.43 ±4.74
November 2015	**C**	29.30 ±4.93	3.01 ±0.74	3.32 ±0.27	29.18 ±5.84
	T	26.39 ±4.05	2.35 ±0.68	3.31 ±0.27	21.87 ±5.46
December 2015	**C**	31.43 ±5.97	3.37 ±0.61	3.41 ±0.28	28.19 ±5.33
	T	31.34 ±5.67	2.92 ±0.74	3.34 ±0.25	21.88 ±6.21

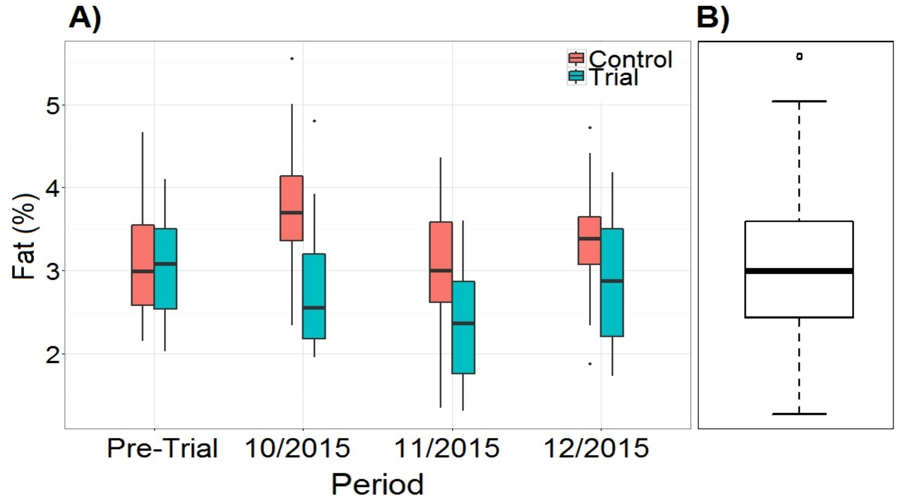

Figure 1 Differences of milk urea level ($mg.100mL^{-1}$) in the trial and control group during whole entire period.

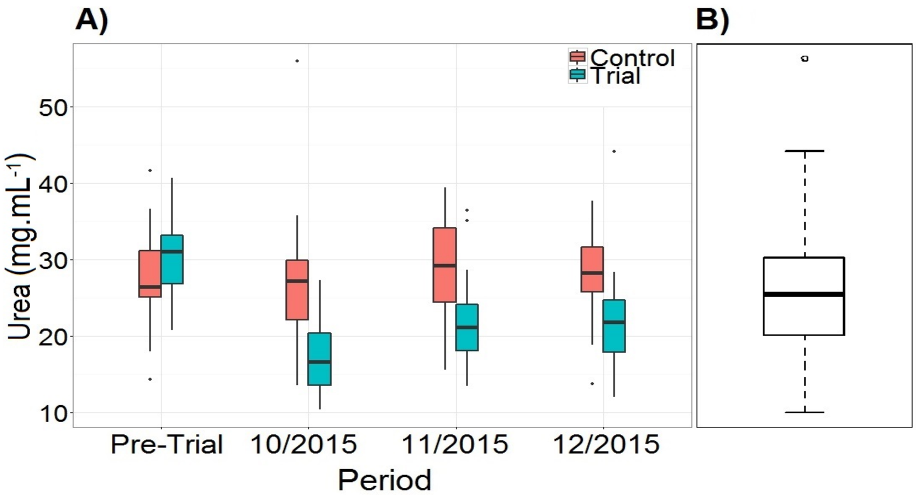

Figure 2 Differences of fat content (%) in the trial and control group during whole entire period.

Statistically significant depression in trial group was found for fat content (0.45%). Highly significant lower urea level was observed in trial group in December 2015 (6.22 mg.mL^{-1}).

After trial period of 3 months, calving rate was calculated as ratio of pregnant cows in the particular group. In the control group half of the cows, whereas in the trial group 68.97% of cows stayed pregnant.

According to observed results, diets with higher concentration of n-3 PUFA cause lower levels of urea in milk. **Thanh and Suksombat (2015)** detected tendency of lower concentration of Urea N in milk after feeding of linseed, sunflower and fish oils in different rations. In comparison with control group the amount of Urea N in milk after feeding of linseed and fish oils lower 1:1 (-2.51mg.100 mL^{-1}), sunflower and fish oils 1:1 (-2.58mg.100 mL^{-1}) and mix of oils (linseed, sunflower and fish oils) 1:1:1 (-2.27 mg.100 mL^{-1}) was lower.

Higher intake of n-3 PUFA caused lower milk production (from -0.39 to -1.39 kg.day^{-1}) in comparison with control group **(Thanh and Suksombat, 2015)**. In contrary, **Bragaglio et al. (2015)** found higher milk yield +1.9 kg.day^{-1} in trial group of animals supplemented with DHA. Then statistically significant decrease in milk protein content from -0.03% to -0.09% was found **(Thanh and Suksombat, 2015)**. Complex of lipids in diets depressed protein (-0.08%) and fat concentration (-0.56%) in milk **(Bodkowski et al., 2016)**. In group of animals with higher concentration of DHA higher fat (+1.27%) and the same milk protein content was found **(Bragaglio et al., 2015)**. Diet enriched on n-3 PUFA reduced milk fat concentration from -0.34% to -1.05% **(Thanh and Suksombat, 2015)**. The milk fat depression is caused by change of ruminal biohydrogenation which leads to the production of different various rumen intermediates and suppress the gene expression of lipogenic enzymes according to **Bauman and Griinari (2001)**.

Our results confirmed that supplementing dairy cow diets with fats containing PUFAs may improve reproductive functions through positive effects on the endocrine system, ovum, embryo, and synthesis of prostaglandins. Significant difference in calving rate (18.97%) was observed between control and trial group. The n-3 fatty acids are likely to improve the survival rate of embryos in cattle. According to study of **Zwyrzykowska and Kupczynski (2016)** the feeding of n-3 PUFA diet tended to increase the number of large follicles and decrease the non-fertilization and/or early embryo mortality rate. Nevertheless, the conception rate at 35 and 90 day after insemination was not affected by diet **(Elis et al., 2016)**. Feeding n-6 PUFA after calving to the first estrous cycle and shifting to n-3 PUFA after the first estrous cycle might be a nutritional strategy to improve reproductive performance and increase the percentage of pregnancies per all inseminations in lactating dairy cows **(Dirandeh et al., 2012)**.

CONCLUSION

The addition of DHA rich algae product (Algae STM Alltech Inc.) had effect on milk production, urea N concentration in milk, milk fat content and pregnant ratio of high yielding Holstein cows. Significant difference (p >0.05) has been observed in level of urea in milk in favour of trial group. In the control group in comparison with trial group lower milk production was found. The feeding of algae caused milk fat depression (p <0.05) and generally lower protein content in milk (p >0.05). Moreover, the results confirmed fact that supplementing dairy cow diets with fats containing PUFAs may improve reproductive functions and can represent effective strategy to increase the percentage of pregnancies per inseminations in lactating dairy cows.

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