# Supplementation of Dairy Cows with Docosahexaenoic Acid Did Not Affect Ovarian Activity

*Martin Vlcek[1], Michaela Andrlikova[1], Olimpia Barbato[2], Vladislav Bina[3], Maurice P. Boland[4], Radovan Dolezel[1], Miloslava Lopatarova[1], Svatopluk Cech[1*]*

[1]*University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic*

[2]*University of Perugia, Perugia, Italy*

[3]*University of Economics, Prague, Czech Republic*

[4]*Alltech, Dunboyne, Ireland*

*\*Corresponding author: cechs@vfu.cz*

## ABSTRACT

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The effect of docosahexaenoic acid (DHA) on ovarian activity of dairy cows was determined. Experimental cows ($n$ = 25) were fed a total mixed ration supplemented daily with 100 g/cow of an algae product All-G-Rich (Alltech, Ireland) containing 10% DHA divided into 2 doses for 52 days. Determination of DHA from milk samples taken from all cows was performed before the All-G-Rich supplementation (on Day 0, D0), and on D21 and D42 of algae supplementation. Cows were synchronized to be in oestrus on D21 and D42 of the experimental period. Monitoring of ovarian activity was performed by transrectal ultrasonography. Examinations were performed at 2–3-day intervals from D0 until D52 of the experimental period. Plasma concentrations of progesterone, oestradiol, insulin, NEFA, and cholesterol were determined. Control cows ($n$ = 25) were examined in the same way as the experimental cows. Milk DHA concentrations on D21 and D42 were significantly higher in treated cows (D21 1.38 vs 0.28, $P < 0.0001$; D42 1.34 vs 0.20, $P < 0.0001$). There were neither important effects of DHA on ovarian structures, nor on evaluated variables in plasma. Cows in the experimental group tended to have larger corpora lutea and higher cholesterol concentrations, but differences were not significant.

**Keywords**: algae; follicle; corpus luteum; oestrus; oestradiol; progesterone; milk; DHA

Lipids present in the feed of dairy cattle are used as an energy source for high yielding cows, however, they constitute important parts of physiological processes. Fatty acids (FA), present in cell membranes as structural phospholipids, influence reproduction in cattle according to the degree of unsaturation and position of the double bonds in the acyl chain of FA (Staples et al. 1998; Mattos et al. 2002). The polyunsaturated FA (PUFA) of the n-6 (linoleic acid) and n-3 (α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA)) families are beneficial for increasing fertility in cows (Santos et al. 2008).

Improvements in dairy cow reproduction with supplemental fat have been associated with improved ovarian function and steroidogenesis, in-

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creased dominant follicle diameter, better oocyte and embryo quality, modulation of prostaglandin synthesis or modification of immune functions of dairy cattle (Garcia-Bojalil et al. 1998; Mattos et al. 2002; Santos et al. 2008). However, data from some studies describing biological action of PUFAs on reproduction in dairy cattle are not always consistent with earlier work (Hutchinson et al. 2012).

The main sources of supplemental fat used in dairy cows are flaxseed, fish oil, fish meal or commercial supplements of FAs containing palmitic acid, conjugated linoleic acid, ALA, and a mixture of EPA and DHA. Marine algae as a dietary source of pure DHA has only rarely been reported (Hostens et al. 2011; Wullepit et al. 2012). More recently Sinedino et al. (2017) have shown that algae increased resumption of oestrous cyclicity (77.6 vs 65.9%) and pregnancy at first artificial insemination (AI) (47.6 vs 32.8%) in primiparous cows. Algae increased pregnancy per AI in all AI in both primiparous and multiparous cows (41.4 vs 30.8%), which reduced days to pregnancy by 21 days compared with control cows.

The aim of the study was to determine the influence of heterotrophically originated DHA (All-G-Rich; Alltech, Ireland), on the ovarian activity of dairy cattle. We examined the hypothesis that DHA from All-G-Rich influences the size of ovarian structures during the oestrous cycle in dairy cows and also influences concentrations of steroid hormones and selected biochemical variables in plasma and follicular fluid in dairy cows.

## MATERIAL AND METHODS

***Animals, treatment, and monitoring***. Animals involved in the study were part of a commercial dairy farm (700 cows) and managed under normal farm conditions. Average milk yield was 9000 kg per year and cows were fed with four types of total mixed ration (TMR) according to the lactational phase. Composition of TMR fed to cows enrolled to the study is shown in Table 1. Cows were fed and milked twice daily.

The study included cows which calved from March to June 2015. Cows ($n$ = 50) were divided equally based on the parity, number of days in milk, and milk yield in the previous lactation into experimental and control groups (Table 2). Both groups

Table 1. Composition of total mixed ration fed to experimental and control group

| Item | |
| --- | --- |
| Corn silage (kg) | 30 |
| Alfalfa haylage (kg) | 4 |
| Rye haylage (kg) | 5 |
| Straw cut (kg) | 0.6 |
| Draff (kg) | 5 |
| Sugar beet pulp (kg) | 5 |
| Molasses (kg) | 0.5 |
| Protein concentrate (kg) | 5.5 |
| Cereals (kg) | 3.5 |
| Limestone (kg) | 0.15 |
| Dry matter (kg) | 24 |
| Net energy of lactation (MJ/kg) | 6.9 |
| Crude protein (%) | 15.5 |
| Starch (%) | 22.8 |
| Neutral detergent fibre (%) | 34.2 |
| Ca (%) | 1.09 |
| P (%) | 0.47 |
| Na (%) | 0.54 |
| K (%) | 1.27 |
| Mg (%) | 0.31 |

were examined and sampled in the same way. A new product All-G-Rich (Alltech, Ireland) based on algae *Schizochytrium* spp. was used as a source of DHA. Powder containing DHA was mixed to TMR in mixer wagon and fed to the experimental cows at a dose of 100 g/cow/day (10 g of DHA) divided into 2 equal doses daily for 54 days.

Presynchronization of cows with cloprostenol (500 µg *pro toto*, Oestrophan® 0.25 mg/ml inj.; Bioveta, Czech Republic) (D1 and D-10 of the experimental period) was performed according to the oestrus detection and rectal palpation in order to achieve day 5–11 of the oestrous cycle of individual cows at D11 of observation (start of synchronization of ovulation – Ovsynch 1). Cows were subjected to

Table 2. Average number of lactations, number of days in milk, and milk yield in experimental and control group at the start of docosahexaenoic acid intake

| | Number of lactations | Days in milk | Milk yield (kg) |
| --- | --- | --- | --- |
| Experimental group | 2.42 ± 1.75 | 47.9 ± 11.4 | 39.2 ± 5.9 |
| Control group | 2.38 ± 1.53 | 47.3 ± 10.5 | 37.7 ± 7.2 |

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the Ovsynch 56-hour protocol. Cows received an intramuscular injection of gonadorelinum (GnRH) (50 µg; Depherelin GONAVET VEYX® inj. ad us. vet.; Veyx-Pharma, Germany) followed 7 days later by an intramuscular injection of cloprostenol (500 µg; Oestrophan®, Bioveta) and a final GnRH injection 56 h after the cloprostenol. Cows were checked for oestrus and examined by ultrasound after each treatment. Cows were synchronized using repeated Ovsynch protocol to be in oestrus on D21 and D42 of the experimental period (Figure 1).

Monitoring of ovarian activity (follicle growth and corpus luteum (CL) development) was performed using an ultrasound Logiq Book (GE Healthcare, USA) equipped with a linear array 7.5–10 MHz transducer. Examinations were performed at 2–3-day intervals from the first synchronized oestrus (D21 of the experimental period) until D53 of the experimental period. All observations were stored in video sequences, ovarian structures were evaluated and measured later. The scanner's tracing function in B mode was used to determine the cross-sectional area (in $cm^2$) of follicles and CLs as previously described (Ginther et al. 2009). For CL with a fluid-filled cavity the area of the cavity was calculated and subtracted from the total cross-sectional area. Calculation of the volume of measured structures was not performed because irregular shapes of CL were often observed.

Two periods of oestrus (Oe 1, D21; Oe 2, D42) and mid points of two luteal phases (LP1, D32; LP2, D53) were the main points monitored and compared in each cow (Figure 1).

***Sampling***. All examinations and samplings were performed at a same time of a day, approximately 6 h after feeding. In all cows, blood samples were taken from a coccygeal blood vessel in 3 different locations in turn (to minimize irritation due to repeated collections) into heparinized tubes (Hemos; Gama, Czech Republic). Samples were immediately centrifuged (3000 *g* for 10 min), plasma was separated and stored at –20°C until assayed. Only blood samples from cows showing single ovulation and bearing one CL in both periods of observation were selected for hormone determination. Blood samples for oestradiol (E2) determination were taken on D1 and D0 of the cycle at the time of the first and second synchronized oestrus (D20, 21 and D41, 42 of the experimental period). Blood samples for progesterone (P4) determination were taken 5× during the first half of oestrous cycle at the time of ovarian activity monitoring during the first and second observed oestrous cycle (D0, D3, D6, D9, and D11 of the cycle; D21–D32, D42–D53 of the experimental period). Blood samples for insulin, cholesterol, and non-esterified FA (NEFA) determination were taken on D21, 32, 42, and 53 of the experimental period (the first synchronized oestrus, D11 of the 1st cycle, the second synchronized oestrus, D11 of the 2nd cycle).

Follicular fluid for E2, P4, insulin-like growth factor 1 (IGF1), insulin, cholesterol, and NEFA determination was aspirated from preovulatory follicles on D42 (oestrus 2) in 10 cows (5 experimental and 5 control cows). Dominant follicles 15–20 mm in diameter were aspirated using a transvaginal ultrasound-guided follicular aspiration (TVFA) according to Cech et al. (2011). The follicular fluid was aspirated manually into a 2 ml plastic syringe until the dominant follicle was aspirated completely. After follicular aspiration cows were synchronized 11 days later, thus, insemination and the final phase of examination were performed 14 days later than in non-aspirated cows.

| Day | –10 | | 0 | 1 | | 11 | | 18 | | 20 | 21 | | 32 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment | Pg | | | Pg | | GnRH | | Pg | | GnRH | | | |
| Event | | | | | | | | | | | Oe 1 | | LP 1 |
| DHA | | | start | | | | | | | | | | |

| Day | 32 | | 39 | | 41 | 42 | | 53 |
|---|---|---|---|---|---|---|---|---|
| Treatment | GnRH | | Pg | | GnRH | | | |
| Event | | | | | | Oe 2 | | LP 2 |
| DHA | | | | | | | finish | |

Figure 1. The experimental schedule

DHA = docosahexaenoic acid, Pg = cloprostenol, Oe 1 = oestrus 1 at day 21, LP 1 = luteal phase 1 at day 32, GnRH = gonadorelin, Oe 2 = oestrus 2 at day 42, LP 2 = luteal phase 2 at day 53

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All procedures were approved by the Institutional Animal Care Committee (trial number PP 6-2015).

*Laboratory analyses.* Determination of DHA from milk samples taken from all cows was performed before All-G-Rich supplementation (D0), and on D21 and D42. First a lipid fraction was separated using a double centrifugation method. Then 0.100 g of the separated lipids were used for conventional transmethylation to produce fatty acid methyl esters (FAME). They were then extracted into n-hexane and quantified using a Gas Chromatograph 6890 (Agilent Technologies, USA) with a flame ionization detector. FAME profiles of each sample were determined by a duplicate injection of 1 µl under these measurement conditions: split ratio 50 : 1, helium carrier gas, flow 1.2 ml/min, particle velocity 20 cm/s, capillary column HP-88 (Agilent Technologies), 100 m, 0.25 mm ID, 0.2 µm film; inlet temperature 220°C, detector temperature 240°C. Peaks were identified using FAMQ-005 (AccuStandard, USA), Reference Standard containing 37 FAME compounds in dichloromethane (equivalent to Supelco FAME Mix 37 standard).

Plasma progesterone, insulin, estradiol, and cortisol were analyzed by RIA and IRMA methods counted in a gamma counter (Packard Cobra II Auto Gamma; PerkinElmer, USA) and determined using commercially available kits according to manufacturers' instructions (progesterone RIA KIP 1458, DIAsource ImmunoAssays SA, Belgium; insulin Irma IM 3210, Beckman Coulter, USA; estradiol A 21854 and cortisol IM 1841 RIA, Beckman Coulter). The inter- and intra-assay coefficients of variation for progesterone, insulin, estradiol, cortisol assays were 6.2 and 8.9%, 5.5 and 9.2%, 6.0 and 7.7%, 5.4 and 8.6%, respectively.

NEFA and cholesterol analyses from the sera samples were studied using diagnostic test kits (NEFA-HR (2), Wako Chemicals GmbH, Germany; cholesterol Randox Laboratories Ltd, UK) running with the principle of enzymatic-colorimetric test. Tests were performed by an automatic biochemistry analyzer (150-20 Spectrophotometer, Hitachi, Japan) under the direction of the kit's manufacturer.

*Statistical analysis.* Basic characteristics (mean, standard deviation, minimum, maximum, and limits of corresponding 95% confidence interval for mean) were computed for all measured variables in both experimental and control groups and for each stage (sample). Both independent groups

Table 3. Milk docosahexaenoic acid (DHA) concentrations in experimental and control group (mg/g of milk fat)]

| Stage | All-G-Rich (mean ± SD) | Control (mean ± SD) | Difference ($P$) |
|---|---|---|---|
| D0 | 0.26 ± 0.40 | 0.35 ± 0.41 | 0.51 |
| D21 | 1.38 ± 0.24 | 0.11 ± 0.28 | 0.0001 |
| D42 | 1.34 ± 0.30 | 0.20 ± 0.37 | 0.0001 |

All-G-Rich = algae product containing 10% DHA (Alltech, Ireland); D0, D21, D42 = days of the experimental period

were compared using Student's two-sample *t*-test (Welch's variant for unequal variances), resulting *P*-values were computed and interpreted.

## RESULTS

Milk DHA concentrations on D0 were similar between experimental and control group. Milk DHA concentrations on D21 and D42 were significantly higher in the experimental group ($P < 0.0001$, Table 3).

The size of the preovulatory follicle on D21 (oestrus 1, D0 of the oestrous cycle) and D42 (oestrus 2, D0 of the oestrous cycle) of the experimental period was similar. The size of CL on D32 (luteal phase 1, D11 of the oestrous cycle) was significantly higher in the experimental group ($P = 0.04$). Also the size of CL on D53 (luteal phase 2, D11 of the oestrous cycle) tended to be larger

Table 4. Size of ovarian structures (cross-sectional area in cm$^2$)

| Stage | All-G-Rich (mean ± SD) | Control (mean ± SD) | Difference ($P$) |
|---|---|---|---|
| F1 | 2.20 ± 0.47 | 2.13 ± 0.63 | 0.34 |
| CL1 | 4.81 ± 1.06 | 4.28 ± 0.93 | 0.04 |
| F2 | 2.07 ± 0.49 | 2.31 ± 0.77 | 0.86 |
| CL2 | 4.70 ± 1.06 | 4.21 ± 0.82 | 0.06 |

All-G-Rich = algae product containing 10% DHA (Alltech, Ireland); D = day, F1 = preovulatory follicle at the first synchronized oestrus on D21 of the experimental period (oestrus 1, D0 of the oestrous cycle), CL1 = corpus luteum on D32 of the experimental period (luteal phase 1, D11 of the oestrous cycle), F2 = preovulatory follicle at the second synchronized oestrus on D42 of the experimental period (oestrus 2, D0 of the oestrous cycle), CL2 = corpus luteum on D53 of the experimental period (luteal phase 2, D11 of the oestrous cycle)

Table 5. Plasma progesterone (P4) concentrations in experimental and control group (ng/ml)

| Sampling | Day of oestrous cycle | Stage | All-G-Rich (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|---|---|
| 1 | 0 | Oe 1 | 1.17 ± 0.16 | 1.19 ± 0.17 | 0.82 |
| 2 | 3 | | 1.88 ± 0.35 | 2.08 ± 0.55 | 0.32 |
| 3 | 6 | | 2.60 ± 0.58 | 2.75 ± 0.80 | 0.62 |
| 4 | 9 | | 4.02 ± 1.56 | 3.50 ± 0.69 | 0.30 |
| 5 | 11 | LP 1 | 5.85 ± 2.83 | 5.25 ± 1.81 | 0.55 |
| 6 | 0 | Oe 2 | 1.44 ± 0.27 | 1.24 ± 0.11 | 0.03 |
| 7 | 3 | | 1.83 ± 0.42 | 1.77 ± 0.51 | 0.76 |
| 8 | 6 | | 2.99 ± 0.81 | 4.18 ± 3.97 | 0.38 |
| 9 | 9 | | 4.01 ± 1.13 | 3.78 ± 1.34 | 0.67 |
| 10 | 11 | LP 2 | 5.88 ± 1.90 | 5.71 ± 2.23 | 0.84 |

All-G-Rich = algae product containing 10% DHA (Alltech, Ireland); D = day, Oe 1 = oestrus 1 at D21, LP 1 = luteal phase 1 (D11 of oestrous cycle) at D32, Oe 2 = oestrus 2 at D42, LP 2 = luteal phase 2 (D11 of oestrous cycle) at D53

in the experimental group, however, the difference was not significant ($P = 0.06$, Table 4).

Progesterone concentrations at the second synchronized oestrus on D42 were significantly higher in the experimental group ($P = 0.03$). Progesterone concentrations at other samplings did not differ between groups (Table 5).

No significant differences were detected in plasma concentrations of oestradiol on D21 (the 1st oestrus) and D42 (the 2nd oestrus) or insulin on D21, 31, 42, and 52 as well as in plasma concentrations of cholesterol on D21, 31, 42, and 52 and NEFA on the same days of the experimental period. Only plasma cholesterol concentration tended to be higher in experimental animals, however, differences were not significant (Tables 6–10).

Likewise, no significant differences in progesterone, oestradiol, insulin, cholesterol, and NEFA concentrations in follicular fluid on D42 (the 2nd oestrus) were found in the experimental vs the control group (Table 10).

Table 6. Plasma oestradiol (E2) concentrations in experimental and control group (pg/ml)

| Stage | Experiment (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|
| Oe 1 | 16.60 ± 9.15 | 16.71 ± 3.63 | 0.97 |
| Oe 2 | 18.45 ± 7.71 | 15.50 ± 5.60 | 0.30 |

D = day, Oe 1 = oestrus 1 at D21, Oe 2 = oestrus 2 at D42

Table 7. Plasma insulin concentrations in experimental and control group (ng/ml)

| Sampling | Stage | Experiment (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|---|
| 1 | Oe 1 | 8.80 ± 3.67 | 9.89 ± 2.99 | 0.44 |
| 2 | LP 1 | 13.33 ± 9.78 | 10.90 ± 5.49 | 0.46 |
| 3 | Oe 1 | 11.25 ± 6.03 | 9.30 ± 2.98 | 0.32 |
| 4 | LP 2 | 11.43 ± 7.82 | 9.21 ± 3.11 | 0.36 |

D = day, Oe 1 = oestrus 1 at D21, LP 1 = luteal phase 1 (D11 of oestrous cycle) at D32, Oe 2 = oestrus 2 at D42, LP 2 = luteal phase 2 (D11 of oestrous cycle) at D53

## DISCUSSION

The main findings from this study were that cows supplemented with DHA that originated heterotrophically (All-G-Rich, Alltech) showed significantly higher concentrations of milk DHA

Table 8. Plasma cholesterol concentrations in experimental and control group (mg/dl)

| Sampling | Stage | Experiment (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|---|
| 1 | Oe 1 | 158.63 ± 39.52 | 135.48 ± 32.08 | 0.14 |
| 2 | LP 1 | 145.53 ± 34.38 | 128.01 ± 40.66 | 0.29 |
| 3 | Oe 1 | 145.04 ± 32.13 | 115.73 ± 46.36 | 0.11 |
| 4 | LP 2 | 133.33 ± 47.70 | 127.63 ± 55.09 | 0.80 |

D = day, Oe 1 = oestrus 1 at D21, LP 1 = luteal phase 1 (D11 of oestrous cycle) at D32, Oe 2 = oestrus 2 at D42, LP 2 = luteal phase 2 (D11 of oestrous cycle) at D53

Table 9. Plasma non-esterified fatty acid concentrations in experimental and control group (mmol/l)

| Sampling | Stage | Experiment (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|---|
| 1 | Oe 1 | 0.25 ± 0.07 | 0.20 ± 0.17 | 0.38 |
| 2 | LP 1 | 0.10 ± 0.06 | 0.07 ± 0.07 | 0.33 |
| 3 | Oe 1 | 0.15 ± 0.07 | 0.13 ± 0.11 | 0.58 |
| 4 | LP 2 | 0.10 ± 0.10 | 0.16 ± 0.12 | 0.21 |

D = day, Oe 1 = oestrus 1 at D21, LP 1 = luteal phase 1 (D11 of oestrous cycle) at D32, Oe 2 = oestrus 2 at D42, LP 2 = luteal phase 2 (D11 of oestrous cycle) at D53

Table 10. Concentrations of progesterone (P4), oestradiol (E2), insulin, cholesterol, and non-esterified fatty acid (NEFA) in follicular fluid of preovulatory follicles on D42 of experiment

|  | Experiment (mean ± SD) | Control (mean ± SD) | P |
|---|---|---|---|
| P4 (ng/ml) | 191.8 ± 62.6 | 266.8 ± 149.1 | 0.3 |
| E2 (ng/ml) | 9.0 ± 1.6 | 6.2 ± 5.8 | 0.3 |
| Insulin (ng/ml) | 3.5 ± 0.9 | 3.9 ± 1.3 | 0.5 |
| Cholesterol (mg/dl) | 18.2 ± 14.5 | 25.3 ± 27.6 | 0.6 |
| NEFA (mmol/l) | 0.2 ± 0.0 | 0.2 ± 0.1 | 0.6 |

in comparison to control cows. There were neither important effects of DHA intake on ovarian structures, nor on evaluated variables in plasma.

Additional fat has been used in dairy cattle for many years as an energy source in TMR and recently, as a source of specifically acting substances. The use of fat in the diets of dairy cattle improves lactation and reproduction when improvements in reproduction occur in spite of provision of calories (Staples et al. 1998). Microbial activity in the rumen results in lipolysis of triacylglycerols and biohydrogenation of PUFA, which reduces the amount of PUFA reaching the small intestine for absorption (Santos et al. 2008). It has been demonstrated that more than 70% of the C18:2 n-6 and more than 85% of C18:3 n-3 PUFAs fed to lactating cows were biohydrogenated in the rumen when fed as unprotected oil or as Ca salts of long chain FA (Juchem 2007).

Despite ruminal biohydrogenation, continual feeding of fat enriched with fish oil (FO) increased the concentrations of EPA and DHA in endometrium (associated with reduction in the proportion of arachidonic acid), liver, mammary gland, muscle, subcutaneous and internal adipose tissues (Bilby et al. 2006b) of dairy cows. However, at the time of start of our study feeding of pure DHA derived from algae has only been mentioned once in a single study (Axman et al. 2015) when the effects of feeding microalgae on growth performance, carcass traits, and FA composition in plasma of finishing heifers were investigated. DHA concentrations in the plasma of supplemented animals increased significantly compared to the control. There was also a linear increase in plasma DHA concentrations as supplementation levels of algae increased.

Therefore, the first aim of our study was the determination of DHA in milk as evidence for the presence of DHA in the body tissues. Milk DHA concentrations on days 21 and 42 were significantly higher in the experimental group. This is in agreement with the articles describing changes in milk reflecting the ration enriched with additional fat of different origin in dairy cattle (Mattos et al. 2002; Bragaglio et al. 2015). We concluded that the dose of 100 g of All-G-Rich is a sufficient dose for peroral administration of DHA.

There are many studies reporting the effects of dietary fats on ovarian follicular activity, function of corpus luteum and steroidogenesis, however, with contradictory results. Older studies often reported the positive influence of additional fat on ovarian activity, increased size of preovulatory follicles, and reduced pregnancy losses (Lucy et al. 1991, 1993; Beam and Butler 1997; Ambrose et al. 2006). Fat supplementation increased the concentration of total cholesterol in follicular fluid; this was associated with the fact that granulosa cells collected from follicles of supplemented cows showed increased progesterone secretion *in vitro* (Wehrman et al. 1991). Ruminants treated with supplemental fat often had small increases in plasma concentrations of progesterone (Staples et al. 1998).

However, not all studies are consistent. Feeding PUFA (18:2, n-6, palm and soybean oil; 18:3, n-3, linseed oil), compared with monounsaturated FA (MUFA) (18:1*c*, n-9 *cis*, sunflower oil; 18:1*t*, n-9 *trans*, mixture of Ca salts of *trans* FA), failed to affect oocyte quality, as demonstrated by subsequent embryo development (Bilby et al. 2006a). Cows fed 18:2- or 18:3-enriched diet had larger preovulatory follicles at insemination and larger

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subsequent volume of the corpus luteum compared with those fed *cis* 18:1 or *trans* 18:1 diets. However, diet did not influence the concentrations of plasma progesterone, insulin, and growth hormone. Authors concluded that the previously documented benefits of PUFAs on reproductive performance appear to reflect actions at an alternative biological window in lactating dairy cows (Bilby et al. 2006a).

Similarly, in a more recent study (Hutchinson et al. 2012) diverse effects of the fat supplements on reproductive parameters were described. Peak plasma oestradiol concentrations were higher in cows receiving flaxseed in comparison to cows receiving FO. Plasma progesterone concentrations were increased in control and CLA-supplemented cows in comparison with cows receiving n-3 PUFA supplements. Authors concluded that feeding n-3 PUFA can have negative implications for embryo development, because of suppressive effects on plasma progesterone concentrations. Even though it has support in an *in vitro* study (Hinckley et al. 1996), where a decrease of the progesterone secretion after incubation of disperse bovine luteal cells with EPA and DHA was observed, such conclusion has never been confirmed. In another study, feeding increasing doses of fish meal and FO containing EPA and DHA did not affect plasma progesterone concentrations in lactating dairy cows (Mattos et al. 2002). In contrast to the study by Hutchinson et al. (2012), the effect of diets enriched in unsaturated FA was shown to enhance early embryonic development in another study (Thangavelu et al. 2007). Progesterone and oestradiol concentrations, as well as follicle growth rate and CL diameter were similar for a group fed FO or control (Childs et al. 2008a). Similarly, a diet enriched with n-3 PUFAs did not affect plasma metabolites, insulin or IGF-1 concentrations, superovulation rate, embryo recovery rate, embryo quality, and expression of the genes associated with embryo development (Childs et al. 2008b).

These studies indicate that various FAs have differential effects on reproductive performance. Understanding which FAs have beneficial effects may permit the feeding of diets enriched in certain FA(s) to enhance fertility (Bilby et al. 2006a). This is in clear agreement with the aim of our study – investigation of the biological action of the single DHA in dairy cattle.

However, we did not observe important significant differences in any selected variables in our study. The size of preovulatory follicles was not different between the groups, this is in agreement with the studies of Childs et al. (2008a) and Hutchinson et al. (2012), but not with that of Bilby et al. (2006a). The size of CL on D11 of the oestrous cycle tended to be larger (on D32 of the experimental period significant difference, on D53 closely nonsignificant), this is in agreement with the study of Bilby et al. (2006a) but not with others (Childs et al. 2008a; Hutchinson et al. 2012). The tendency for higher cholesterol concentrations determined in all terms in our study is in agreement with previous studies (Bilby et al. 2006a; Childs et al. 2008a; Hutchinson et al. 2012), however, nonsignificant. It could be due to the total amount of fat supplement fed to the animals in our study. Total lipid supplement intake differs among studies: 200 g/day (Mattos et al. 2004), 500 g/day (Hutchinson et al. 2012), 1.75% of dietary dry matter (DM) (Bilby et al. 2006a), up to 7.8% of DM (Mattos et al. 2002). In our study, 0.1 kg of All-G-Rich daily represents only ca. 0.5% of dietary DM, so a possible positive effect on energy balance can be excluded. Plasma concentration of steroid hormones, except progesterone, at the time of the second oestrus did not differ between groups. Plasma insulin as well as NEFA concentrations did not differ between the experimental and control groups in our study. Those results are consistent with the majority of studies, which concluded that feeding n-3 PUFAs did not increase plasma steroids concentrations (Mattos et al. 2002; Bilby et al. 2006a; Childs et al. 2008a; Hutchinson et al. 2012).

In a recent meta-analysis (Rodney et al. 2015), authors concluded that feeding fats had a positive effect on fertility and had a tendency to increase production when given during the transition period. However, meta-regression of the difference in diets between treatment and control groups did not identify the reasons for these improvements in regard to the FA composition of the diet. The limited number of papers found and the positive results of this study support the need for further tasks exploring the effects of fat supplementation of the diet of the transition cow on fertility and the development of guidelines to assist study design in this field of research (Rodney et al. 2015).

We concluded that feeding the DHA originating from *Schizochytrium* spp. (All-G-Rich, Alltech) did

not influence ovarian activity in dairy cows in our study. Positive effects of All-G-Rich on fertility, as recently demonstrated in a large trial (Sinedino et al. 2017), should be investigated in different areas of reproductive physiology and this may require further research.

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