Effects of a n-3 polyunsaturated fatty acid-enriched diet on embryo production in dairy cows

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Title:

Effects of a n-3 polyunsaturated fatty acid enriched diet on embryo production in dairy cows

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Abstract (250 words)

Beneficial effects of n-3 polyunsaturated fatty acid (PUFA) supplementation on dairy cow reproduction have been previously reported. The objectives of the present study were to assess whether n-3 PUFA supplementation would affect *in vitro* embryo production (IVP) after ovarian stimulation. Holstein cows received a diet with 1% dry matter supplementation of either n-3 PUFA (n = 18, micro encapsulated fish oil) or a control, n-6 PUFA (n = 19, micro encapsulated soy oil). Both plasma and follicular fluid FA composition showed integration of total PUFA through the diet. All cows underwent an IVP protocol consisting of ovarian stimulation, ultrasound-guided transvaginal oocyte retrieval (ovum pick-up, OPU, 5 per cow) followed by *in vitro* maturation, fertilisation and 7 days of embryo development. A tendency toward an increase in the blastocyst rate (diet effect, p = 0.0865) was observed in n-3 cows, with 49.6 ± 5.5%, versus 42.3 ± 5.5% in control n-6 cows. A significant increase (diet effect, p = 0.0217) in the good quality blastocyst rate (freezable blastocysts) was reported in n-3 cows (42.2 ± 7.7%) compared to control n-6 cows (32.7 ± 7.7%). A significant difference in lipid composition was shown in the oocytes recovered by OPU from n-3 and n-6 treated cows, by intact single-oocyte MALDI-TOF mass spectrometry. The 42 differentially abundant identified lipids were mainly involved in cell membrane structure. In conclusion, n-3 PUFA supplementation enhanced oocyte quality and modified their lipid composition. Further studies are necessary to investigate the potential link of these lipid modifications with enhanced oocyte quality.

Introduction

Reproductive performance in cows is influenced by nutritional and metabolic status, as previously reviewed (Butler, 2000, Roche, 2006, Leroy et al., 2008). Appropriate plasma fatty acids (FA) and polyunsaturated FA (PUFA) composition affects various reproductive processes, including steroid hormones and prostaglandin precursor syntheses via cholesterol and arachidonic acid, respectively (Urlep et al., 2013, Tessaro et al., 2015). Previous studies reported beneficial effects of n-3 PUFA supplementation on dairy cow reproduction (reviewed in (Moallem, 2018). The n-3 PUFA belong to a family of biologically active essential FA. Given the poor conversion rate between the n-3 PUFA short chain alpha-linolenic acid (ALA, C18:3), and the long chain n-3 PUFA (LC n-3 PUFA), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6), these LC n-3 PUFA need to be provided directly through the diet in order to increase their abundance in the plasma (Simopoulos, 2002, Plourde et al., 2007, Calder, 2012). EPA and DHA are mainly found in fish oil, as well as in microalgae oil (Abughazaleh et al., 2009).

The n-3 PUFA have beneficial effects on cow reproduction, in several ways. Indeed, cows being fed a flaxseed diet had a higher pregnancy rate in relation to a decrease in embryo mortality (Petit et al., 2006), as well as an increase in the size of pre-ovulatory (Ambrose et al., 2006) and small follicles (Zachut et al., 2010). Conception rates also tended to be higher in cows receiving an ALA-enriched diet (Ambrose et al., 2006, Dirandeh et al., 2013) or a LC n-3 PUFA diet (Sinedino et al., 2017, Elis et al., 2016b). One reported mechanism leading to increased conception rates was the uterine effect of n-3 PUFA reducing prostaglandin F2 alpha (PGF2α) and, therefore, reducing embryo mortality (Mattos et al., 2004). Nevertheless, other studies suggested a direct beneficial ovarian effect of LC n-3 PUFA. Indeed, *in vitro* embryo production, performed after ultrasound-guided transvaginal oocyte retrieval (ovum pick-up) without ovarian stimulation treatment, was enhanced after n-3 PUFA supplementation compared to the control, consisting of a saturated FA supplementation (Moallem et al., 2013). Moreover, *in vitro* studies also reported that *in vitro* maturation (IVM) medium supplemented with either ALA (Marei et al., 2009) or low dose DHA (Oseikria et al., 2016) led to increased embryo developmental rates. Since previous studies also reported that n-6 PUFA could lead to beneficial effects on reproduction (reviewed in (Leroy et al., 2014), it is therefore important to compare n-3 PUFA supplementation to a n-6 control, in order to prove a

specific n-3 PUFA effect. Indeed, when comparing n-3 PUFA to a saturated control, any beneficial effect could be due to the difference between saturated and polyunsaturated FA, which is not specific to n-3 PUFA.

The present study relies on the hypothesis that n-3 PUFA supplementation of the diet of donor cows could improve embryo production both in terms of number and quality of embryos after ovarian stimulation. The objectives of the present study in dairy cows were therefore 1) to assess whether dietary supplementation with LC n-3 PUFA (in comparison with a n-6 PUFA control diet) would affect *in vitro* embryo production, performed after ovarian stimulation by gonadotrophins (FSH treatment), thus mimicking usual *in vitro* or *in vivo* embryo production conditions; 2) to assess several LC n-3 PUFA supplementation durations in order to determine whether a short duration could be long enough to observe beneficial effects of the diet on embryo production; and 3) to analyse oocyte lipid composition after LC n-3 PUFA diet supplementation.

Materials and methods

Experimental design

All experimental protocols were conducted in accordance with the European Directive 2010/63/EU on the protection of animals used for scientific purposes and approved by the French Ministry of National Education, Higher Education, Research and Innovation after ethical assessment by the local ethics committee "Comité d'Ethique en Expérimentation Animale Val de Loire (CEEA VdL)" (protocol registered under ref. APAFIS#2098-2015100115278976_v5).

Holstein primiparous cows (n = 37) were managed in loose housing during lactation. In order to conclude that the observed effects were specifically due to n-3 PUFA, cows supplemented with n-6 PUFA were used as a control group. Each cow received either n-3 (n = 18) or n-6 (n = 19) supplementation for 9 weeks, starting at ≈11 weeks postpartum. All cows underwent five sessions of OPU. Three of these OPU sessions, occurring after ovarian stimulation (FSH treatment), led to *in vitro* embryo production, after 2, 5 and 7 weeks of supplementation. The two other OPU sessions led to oocyte collection and lipidomic analyses, after 3 weeks (without ovarian stimulation) and 9 weeks (with ovarian stimulation) of supplementation (Figure 1).

Experimental diets and dry matter intake

For 9 weeks, dairy cows received a diet consisting of (% of total diet dry matter, DM): 59.4% corn silage, 14.5% Sandilait® (soybean and rapeseed meal; Agralys, Blois, France), 9.5% Sandifort® (wheat; Agralys, Blois, France), 8.5% alfalfa hay, 7.5% dehydrated alfalfa, 0.2% vitamins and minerals 5/23, 0.4% calcium bicarbonate. The n-3 PUFA supplement was OMG750®, a microencapsulated fish oil (Kemin, Nantes, France) and the n-6 PUFA supplement was OMGSOY®, a microencapsulated soy oil (Kemin, Nantes, France), with both supplements being distributed at 1% DM. The two diets were total mixed rations (TMR), with feed values calculated using the INRA French feeding system (INRA, 2018). The supplements were manually mixed into the diet and the TMR were distributed twice daily in individual weighing troughs (Insentec B.V., Marknesse, The Netherlands), as described by (Elis et al., 2016b). Cows were fed *ad libitum*

and DM intake (DMI) was determined from the feed intake, the composition of the diets and the dry matter content of each ingredient included in the diets. The nutritional values of the different ingredient compositions in the diets were calculated from a chemical analysis. DMI was analysed daily during the distribution of the n-3 or n-6 diets, throughout the supplementation period.

Body weight, milk yield and energy balance

Body weight (BW) and milk yield (MY) were monitored daily during the supplementation period. All cows were milked twice daily. In the milking parlor, the MY (kg/d) of each cow was automatically recorded (software Manufeed 500 pro, vc5 version 2.011.14). After each milking period, cows were automatically weighed (software RIC version RW1.7). Only the BW recorded in the morning was used for statistical analysis.

Energy balance (EB, expressed in Mcal/d) was calculated during the supplementation period and corresponds to the difference between net energy intake and net energy needs for body maintenance and lactation, according to the INRA method (INRA, 2018).

AMH and metabolic assays, fatty acid composition of plasma and follicular fluid

Plasma Anti Müllerian Hormone (AMH) was determined using the AMH Gen II ELISA assay (Beckman Coulter, Villepinte, France) using 50 µL of undiluted plasma, as previously described (Rico et al., 2009), from samples collected the first day of the diet supplementation and stored at -20 °C. AMH, an indicator of the ovarian response after hormonal stimulation, was assessed for any differences in plasma AMH between the groups.

For metabolic assays, plasma samples were collected once per week from caudal venipuncture before feeding, during the supplementation period. Plasma samples were stored at -20 °C until the assays were conducted. Plasma NEFA, urea and glucose were determined using enzymatic colorimetry with a multi-parameter analyser (KONE Instruments Corporation, Espoo, Finland).

Plasma and follicular fluid fatty acid compositions were determined using gas chromatography, as previously described (Lefils et al., 2010). Plasma fatty acid composition was determined at the beginning of the supplementation period and in samples corresponding to OPU leading to *in vitro* embryo production (at 2, 5 and 7 weeks postpartum, Figure 1). Follicular fluid fatty acid composition was determined at 2, 5 and 7 weeks postpartum.

Embryo production

Oestrus synchronisation and ovarian stimulation

Eleven days prior to supplementation (=d0, Figure 1), each cow received a synchronisation treatment consisting of a 3.3 mg norgestomet subcutaneous implant and an intra muscular (IM) injection of 0.001 mg buserelin (Crestar Pack®, MSD Santé Animale, Beaucouzé, France). Seven days later, each cow received an IM injection of 15 mg luprostriol (Prosolvin®, Virbac, Carros, France) and 2 days later, the subcutaneous implant was removed. Oestrus was observed ≈48 h following implant removal, which corresponded to the first day of supplementation, and a new subcutaneous implant of 3.3 mg norgestomet was inserted this same day (Crestar® SO, MSD Santé Animale, Beaucouzé, France). The norgestomet implant was then replaced every 10 days during the 9 weeks of the experiment, until the end of the protocol, to prevent the occurrence of oestrus between OPU sessions.

Ovarian stimulation was performed using 400 µg of pFSH per cow (Stimufol®, Reprobiol, Ouffet, Belgium) divided into five IM injections of decreasing FSH doses (112, 100, 75, 63 and 50 µg) every 12 h, beginning 60 h prior to the follicular punctures.

Ovum pick-up

The ultrasound-guided transvaginal oocyte retrieval (ovum pick-up) was performed under locoregional anaesthesia (epidural block) with 86.5 to 173 mg of procaine per cow (Procamidor®, Richter Pharma, Wels, Austria), after sedation with 10 mg/100 kg xylazine (Rompun®, Bayer Animal Health, Saint-Georges-de-Reneins, France). The anogenital area was then washed and disinfected (Vétédine savon®, Vétoquinol,

Lure, France). A guide containing an ultrasonographic probe was inserted via the vaginal route and the ovary was positioned by the technician through the transrectal route against the probe (probe EC123, 7.5 MHz, echograph MyLab30, ESAOTE Pie Medical, Saint-Germain-en-Laye, France). A needle holder, including a needle (18 G) and linked to a suction system, was introduced via the transvaginal route. The 37 cows were divided into five groups of 6 to 9 cows. For each group, one OPU session occurred on the same day. The complete experimental design (groups 1 to 5) was performed over a 14-month period.

In vitro embryo production

Complexes oocyte-cumulus (COC) recovered from OPU sessions, performed after 2, 5 and 7 weeks of supplementation, were selected and underwent *in vitro* maturation (IVM) followed by fertilisation (IVF). All straws of semen used to performed the IVF were produced from a single ejaculate of a single Holstein bull. The produced zygotes then underwent *in vitro* development (IVD), according, according to a previously described protocol (Elis et al., 2017, Oseikria et al., 2016). Quality of the produced embryos was assessed through the cleavage rate at 48 h (reported to COC in IVM) and the blastocyst rate (reported to cleaved embryos) at 7 days following IVF. Blastocyst quality was also graded, with grades ranging from quality 1 (Q1) to quality 4 (Q4) blastocysts, according to the IETS (International Embryo Technology Society) morphological criteria (International Embryo Transfer, 2013). Q1 and Q2 blastocysts were considered as good quality and freezable blastocysts. All embryos have been graded by the same lab technician.

Oocyte lipidomic analyses

Immature oocytes recovered from OPU sessions performed after 3 and 9 weeks of supplementation, in 9 (from the 18) n-3 cows and 9 (from the 19) n-6 cows, were used for lipidomic analyses: a total of 60 n-3 oocytes and 61 n-6 oocytes were compared.

Immature oocytes were completely mechanically denuded from Cumulus cells (CC) by repeated pipetting and individually snap-frozen in drops of 20 mM Tris-HCl pH 6.8/260 mM sucrose buffer. Lipid profiles of individual oocytes were obtained using an UltrafleXtreme MALDI-mass spectrometer in positive reflector

mode using 2,5-dihydroxyacetophenone matrix, as described (Elis et al., 2017). M/z peaks were detected in the range of 400 to 1000 m/z and values of the normalised peak heights (NPH) were quantified using Progenesis MALDI™ (Nonlinear Dynamics). Lipids were identified by high-resolution mass spectrometry by LC-MS or by direct infusion combined with top-down MS/MS analyses (Bertevello et al., 2018).

Statistical analyses

Statistical analyses were performed using SAS® software (SAS institute Inc., 2013), unless otherwise indicated. The stages (days in milk) and plasma AMH levels of n-3 and n-6 cows at the beginning of supplementation were compared using a Wilcoxon test (NPAR1WAY procedure). Linear mixed models (MIXED procedure) including the effects of diet, week of supplementation (= rank of OPU session), diet x week of supplementation interaction, with week of supplementation as a repeated effect within cow (repeated statement of the MIXED procedure, an AR(1) covariance structure was used for all parameters except for plasma and follicular fluid FA composition for which a CS covariance structure was used), and a random group effect were used for the following parameters: BW (with BW at 1 week of supplementation as a covariate), MY (with the day in milk corresponding to the first day of supplementation as a covariate), DMI (with BW as a covariate), EB, plasma NEFA, glucose, urea (with, for each of these three parameters, the plasma level measured on the first day of supplementation as a covariate), plasma and follicular fluid FA composition, number of COC recovered and that underwent IVM, number of embryos produced per OPU session. Logistic regression mixed models (GLIMMIX procedure) including the effects of diet, week of supplementation (= rank of OPU session), diet x week of supplementation interaction, and a random group effect were used for the following parameters: cleavage rates and embryo development rates. Results are presented as least squares means (lsmeans) ± SEM, unless otherwise indicated. Multiple comparisons of lsmeans estimated by the models were performed with a T-test, and a Bonferroni adjustement was used for all parameters except COC and embryo production parameters, plasma and follicular fluid FA composition parameters.

Relative lipid abundance in individual oocytes were compared using a T-test with a Benjamini–Hochberg correction applied to the NPH values of each lipid species; multivariate principal component analysis (PCA) was performed using differential NPH values (XLSTAT, Addinsoft, Paris, France).

Effects with $p \leq 0.05$ were considered significant, and effects with $0.05 < p \leq 0.1$ were considered tendencies.

Results

Physiological measurements

Supplementation began at 11.2 ± 0.3 weeks after calving, with no difference between the control n-6 and n-3 groups ($p = 0.9273$). No difference was reported between n-3 and n-6 groups for BW, MY, DMI or EB (Figure 2). During the 9-week supplementation period and the previous 8 weeks, BW averaged 572.3 ± 3.4 kg in n-3 cows *versus* 573.5 ± 3.3 kg in n-6 cows (diet effect, $p = 0.7421$). MY averaged 28.5 ± 1.5 kg/d in n-3 cows *versus* 28.8 ± 1.5 kg/d in n-6 cows (diet effect, $p = 0.8069$). During the 9-week supplementation period, DMI averaged 19.2 ± 1.0 kg/d in n-3 cows compared to 19.4 ± 1.0 kg/d in n-6 cows (diet effect, $p = 0.8912$). EB was, on average, 0.6 ± 1.1 Mcal/d in n-3 cows *versus* 1.2 ± 1.1 Mcal/d in n-6 cows (diet effect, $p = 0.6192$). For these four measurements, the effect of week of supplementation was significant ($p \leq 0.05$), without an interaction between diet and week of supplementation.

Metabolic measurements

Metabolic measurements, measured in plasma (NEFA, glucose, urea), did not differ between n-3 cows and control n-6 cows during the 9-week supplementation period (Figure 3). Indeed, n-3 cows exhibited a plasma NEFA level of 0.30 ± 0.02 mM compared to 0.32 ± 0.02 mM in the control n-6 cows (diet effect, $p = 0.2911$). The plasma glucose levels were, on average, 4.18 ± 0.05 mM in n-3 cows *versus* 4.13 ± 0.05 mM in control n-6 cows (diet effect, $p = 0.4925$). The plasma urea levels were not different (diet effect, $p = 0.1834$) between n-3 cows (4.23 ± 0.17 mM) and control n-6 cows (4.40 ± 0.17 mM). For these three measurements, the effect of the week of supplementation was significant ($p \leq 0.05$), without an interaction between diet and week of supplementation, except for urea level, which had a significant effect of the interaction between diet and week of supplementation ($p = 0.025$).

Plasma and follicular fluid fatty acid composition

The fatty acid composition, expressed in % of total fatty acids, was measured in plasma, to assess the efficiency of the PUFA supplementation (Table 1), and in follicular fluid, to study how PUFA nutritional

changes affect the fatty acid composition of this ovarian compartment (Table 2). Half of the measurements in plasma (15/27) and follicular fluid (10/27) varied throughout the period of supplementation (week of supplementation effect, $p \leq 0.05$). In n-3 cows, after only 2 weeks of supplementation, a significant 2-fold increase was observed in plasma EPA, compared to the period before supplementation ($p < 0.0001$). Similarly, a significant 2.1-fold increase ($p < 0.0001$) and 1.2-fold increase ($p < 0.0001$) were also observed for DHA and total n-3 PUFA, respectively, compared to the period before supplementation. The week of supplementation effect was $p < 0.0001$ for EPA, $p < 0.0001$ for DHA and $p = 0.0033$ for total n-3 PUFA; these increases validated the efficiency of the n-3 diet. Concerning EPA and total n-3 PUFA, an effect of the diet was reported ($p < 0.0001$): plasma level at 2 weeks of supplementation was significantly increased for EPA (1.7-fold, $p < 0.0001$) and total n-3 PUFA (1.2-fold, $p = 0.0002$) in n-3 cows compared to n-6 cows; plasma level at 7 weeks of supplementation was significantly increased for DHA (1.6-fold, $p = 0.0298$), this increase being a tendency at 5 weeks of supplementation (1.5-fold, $p = 0.0534$), in n-3 cows. Moreover, the follicular fluid level also increased by 1.3-fold for EPA after 2 weeks of supplementation ($p = 0.0344$) and for total n-3 PUFA after 5 weeks of supplementation ($p < 0.0001$) in n-3 cows compared to control n-6 cows. Concerning total n-6 PUFA (diet effect, $p = 0.0081$), a tendency toward a 1.1-fold increase was reported in control n-6 cow plasma at 2 weeks of supplementation ($p = 0.0833$), with this 1.1-fold increase becoming significant at 5 weeks of supplementation ($p = 0.0039$). In n-3 cows, the n-6 PUFA/n-3 PUFA ratio (diet effect, $p = 0.0010$) was 1.3-fold lower in n-3 cow plasma at 2 weeks of supplementation ($p = 0.0075$) and 1.6-fold lower in n-3 cow follicular fluid at 7 weeks of supplementation ($p = 0.0052$), compared to control n-6 cows. For these five measurements (EPA, DHA, total n-3 PUFA, total n-6 PUFA and n-6/n-3 ratio), the diet x week of supplementation interaction was significant ($0.0001 < p \leq 0.0012$).

Embryo production

Three embryo production sessions by OPU-IVF were performed for 18 n-3 cows and 19 control n-6 cows, generating 54 and 57 OPU-IVF sessions and a total of 1462 and 1538 punctured follicles, respectively (Table 3). Plasma AMH level was not different between the groups ($p = 0.4941$), suggesting an absence of

a difference in the ovarian response to hormonal stimulation between the groups; it was, on average, 299.0 ± 73.4 pg/mL in n-3 cows compared to 242.3 ± 55.6 pg/mL in control n-6 cows. The average number of punctured follicles per OPU session did not differ (diet effect, $p = 0.8196$) between n-3 cows (26.7 ± 4.3) and control n-6 cows (26.1 ± 4.3).

A significantly higher recovered COC rate (diet effect, $p = 0.0035$) was observed in n-3 cows (38.0 ± 1.6%) compared to control n-6 cows (32.8 ± 1.6%). This rate corresponded to 10.2 ± 1.3 oocytes recovered per OPU session in n-3 cows compared to 8.5 ± 1.3 from control n-6 cows (diet effect, $p = 0.2297$). The cleavage rate tended to be lower ($p = 0.1033$) in n-3 cows (77.3 ± 3.8%) compared to control n-6 cows (82.3 ± 3.3%), which corresponded to 5.8 ± 1.0 cleaved embryos in n-3 cows *versus* 5.0 ± 1.0 in control n-6 cows (diet effect, $p = 0.3597$). The blastocyst rate tended to be higher (diet effect, $p = 0.0865$) in n-3 cows (49.6 ± 5.5%), versus control n-6 cows (42.3 ± 5.5%), corresponding to 2.8 ± 0.6 blastocysts per OPU session in n-3 cows *versus* 2.2 ± 0.6 blastocysts in control n-6 cows (diet effect, $p = 0.2136$). This tendency was observed only during the first OPU session ($p = 0.1024$), therefore, only after 2 weeks of supplementation. The quality of blastocysts was also morphologically graded, into good quality embryos (Q1 and Q2 according to the IETS classification, considered as freezable embryos) and lower quality embryos (Q3 and Q4). A significant increase (diet effect, $p = 0.0217$) in the Q1-Q2 blastocyst rate was noted in n-3 cows (42.2 ± 7.7%) compared to control n-6 cows (32.7 ± 7.7%), which corresponded to 2.5 ± 0.5 Q1-Q2 blastocysts in n-3 cows *versus* 1.8 ± 0.5 Q1-Q2 blastocysts in control n-6 cows (diet effect, $p = 0.1527$). This difference was significant after 5 weeks of supplementation ($p = 0.0447$) and a trend after 2 weeks of supplementation ($p = 0.0773$). No difference was observed after 7 weeks of supplementation between the diet groups, for any parameter. Indeed, in n-3 cows, embryo development rates were maintained at the same levels as for the previous OPU-IVF session (after 2 and 5 weeks of dietary supplementation), but were no longer different from the developmental rates of control n-6 cows. Concerning embryo development rates, no parameter showed neither a significant week of supplementation effect, except for the recovered COC rate ($p = 0.0054$), nor a significant diet x week of supplementation interaction. Concerning the number of embryos produced per OPU session, no parameter showed a significant week of supplementation effect or a significant diet x week of supplementation interaction.

290

291 **Oocyte lipid composition**

292 A total of 60 single-oocytes from 9 n-3 cows (n-3 oocytes) and 61 oocytes from 9 n-6 cows (n-6 oocytes)
293 were analysed by intact cell MALDI-TOF mass spectrometry, to compare their lipid composition.
294 Physiological and metabolic measurements for these two subgroups (9 n-3 and 9 n-6 cows) were
295 representative of the whole groups (respectively 18 n-3 and 19 n-6 cows) (Supplementary Figure 1 and
296 Supplementary Tables 1 and 2). Hierarchical clustering was performed, based on 110 m/z that were
297 differentially abundant between n-3 and n-6 oocytes ($p < 0.05$, > 2-fold change, Figure 4A). A heatmap
298 represents the relative abundance of 55 up-regulated and 55 down-regulated m/z in the n-3 group as
299 compared to the n-6 group (Figure 4A and Supplementary Table 3); each line corresponds to one oocyte
300 and each column corresponds to one differential molecular species. Two clear clusters were distinguished,
301 corresponding to over-abundant lipids in either n-3 or n-6 oocytes. A principle component analysis
302 performed on differential m/z clearly discriminated n-3 oocytes and control n-6 oocytes, based on their
303 lipid composition (Figure 4B). Among these 110 differential m/z, 42 lipid species were identified
304 (Supplementary Table 4). Among them, 12 phosphatidylcholines (PC), three phosphatidylethanolamines
305 (PE, C36), two sphingomyelins (SM, C35) and lyso-phosphatidylcholine LPC 22:4 were more abundant in
306 n-3 oocytes, whereas 15 PC, PE 30:0, SM 34:1, two LPC (16:0 and 18:0) and two triacylglycerols (46:1,
307 47:1) were more abundant in n-6 oocytes.

308

309 **Discussion**

310 This present work is the first, to our knowledge, to assess whether the effects of a n-3 diet on *in vitro* embryo
311 production in dairy cows still occurred after ovarian stimulation, compared to control cows supplemented
312 with a n-6 diet; it was also the first to assess the effects of several supplementation durations. The results
313 suggest that n-3 supplementation could enhance embryo quality, even with a relatively short
314 supplementation duration of 2 to 5 weeks.

315 Supplementation of the diet with n-3 PUFA led to an increase in the Q1-Q2 blastocyst rate, even
316 when compared to control cows supplemented with n-6 PUFA, and even after ovarian stimulation. Such

supplementation could therefore be a benefit to the *in vitro* embryo production field. This increase in good quality embryos is consistent with previous studies reporting a positive effect of n-3 supplementation on conception or gestation rates (for a review, (Moallem, 2018). Indeed, n-3 supplementation seemed to enhance gestation rate (Ambrose et al., 2006, Petit et al., 2006, Dirandeh et al., 2013, Sinedino et al., 2017), partly through enhancing embryo implantation (Mattos et al., 2003), but also by affecting oocyte quality (Elis et al., 2016b, Moallem et al., 2013). Moreover, other studies were performed on embryo production, without ovarian stimulation treatment. An increase in the cleavage rate was reported after flaxseed (Zachut et al., 2010) and fish oil supplementation (Moallem et al., 2013). These results were also consistent with *in vitro* studies in which a supplementation of the IVM medium with n-3 PUFA, therefore over only 24 h, also led to an enhancement of the metaphase2 oocyte rate and the subsequent cleavage and blastocyst rates (Elis et al., 2017, Oseikria et al., 2016, Marei et al., 2009). In the future, it would be interesting to assess whether coupling the supplementation of the diet of oocyte donor cows and of IVM medium with n-3 PUFA could further improve *in vitro* embryo production.

The increases in blastocyst rates reported in the present study were only moderate, but these results are nonetheless interesting, and consistent with another study also performed after ovarian stimulation (Thangavelu et al., 2007). In such conditions, one could expect a loss of this effect, due to the strong ovarian stimulation effect of hormonal treatment. The observation of such an effect of n-3 supplementation is thus an interesting result that is encouraging for its use in embryo production or for the improvement of the fertility of dairy cows. Moreover, the control used in the present study was n-6 supplementation, which is the optimal control to evaluate a specific effect of n-3 PUFA. Indeed, when comparing the effect of n-3 PUFA to a saturated FA control, any reported difference could be due to the fact that a saturated FA is being compared to a PUFA, but it would not be possible to prove that it is due to the specific effect of n-3 PUFA. On the other hand, the n-6 PUFA control exhibited similar effects as n-3 PUFA (Leroy et al., 2014); it is therefore more difficult to prove a significant difference between the n-6 and n-3 groups of cows. Previous studies (cited above) reported that cleavage rates increase in n-3 PUFA groups compared to saturated controls (Zachut et al., 2010, Moallem et al., 2013), but they also reported an absence of a difference when compared to a n-6 PUFA group (Zachut et al., 2010). Moreover, another study reported a

difference in the number of blastomeres of transferable embryo after PUFA supplementation compared to saturated supplementation, but it was not possible to highlight such difference between n-3 and n-6 supplementation group (Thangavelu et al., 2007).

In the present study, no difference in the number of punctured follicles was shown. This was expected because, even if n-3 PUFA supplementation has been reported to increase follicular population (Petit et al., 2002, Bilby et al., 2006, Zachut et al., 2010), this same effect is also observed with n-6 PUFA supplementation (for a review, (Leroy et al., 2014). Moreover, none of the embryo production parameters measured at the fourth OPU (after 7 weeks of supplementation) were different between n-3 PUFA and n-6 PUFA cows. Indeed, after 7 weeks of supplementation, a significant increase in plasma n-6 PUFA was reported in the FA composition of the control n-6 compared to the n-3 PUFA cows. This increase might suggest that the beneficial effects of n-6 PUFA on embryo production could explain why control n-6 PUFA embryo rates matched the embryo rates of the n-3 PUFA group, rendering it impossible to find a difference between the groups. Even though the addition of a third experimental group of cows (consisting of a saturated FA diet as a control) was not possible in our conditions, a comparison with this third group would have highlighted the obtained results.

Concerning the duration of n-3 PUFA supplementation required to observe a beneficial effect, the present study suggests, even if it has to be further confirmed, that a short duration of supplementation (around 2 to 5 weeks) could be long enough to observe an improvement in embryo production. Indeed, in our study, a longer period of supplementation did not lead to higher embryo rates. A short period of supplementation means that such supplementation could be applied to the field of embryo production, by limiting the cost of supplementation (3.6€/d/cow in average in our study). Moreover, *in vitro* results suggested that improvement of blastocyst rates was especially observed in low quality oocytes (Elis et al., 2017, Oseikria et al., 2016); it is therefore possible that such improvements would not be observed in high quality oocytes. In addition, the positive effects on embryo rates have not always been reported, i.e. when working with oocyte donor heifers rather than cows in lactation (Ponter et al., 2012). Discrepancies could be attributed to differences in the amount or form of PUFA supplementation, but might also be attributed

to the quality of the recovered oocytes. Therefore, the type of supplementation would be especially recommended when working with high genetic merit cows with unpredictable oocyte quality.

The increases in EPA, DHA and total n-3 PUFA percentages in plasma and/or follicular fluid fatty acid composition allowed for the validation of the efficiency of such supplementation in dairy cows, which is in line with our previous study (Elis et al., 2016b). The present study also showed that fatty acid composition variations observed in the plasma were similar to variations observed in the follicular fluid, which is consistent with the literature (Childs et al., 2008, Moallem et al., 2013). These variations needed a longer period of supplementation to appear in follicular fluid compared to plasma, where they were demonstrated after 2 weeks of supplementation.

To ensure that our results were not biased, we also studied physiological measurements. Indeed, differences in MY or EB could contribute to differences in embryo quality. In our study, n-3 PUFA supplementation was not associated with differences in BW, MY, DMI or EB nor with metabolic differences in plasma NEFA, glucose or urea between the two groups of cows; this is consistent with our previous data reporting the effect of this type of diet in dairy cows (Elis et al., 2016b). Indeed, the supplementation was isoenergetic between n-3 and control n-6 groups, and moderate (1% DM), and we therefore did not report the physiological variations that could be observed with supplementations at higher doses or in a different form, such as a reduction in DMI and an increase in MY (Leroy et al., 2014). The absence of metabolic differences, which are in line with previous studies for NEFA (Mattos et al., 2004, Mashek et al., 2005) and plasma glucose (Ambrose et al., 2006) levels, are also probably due to the moderate quantity (1% DM) distributed.

Finally, an increase in the recovered COC rate was reported in n-3 PUFA cows. Even if we have no direct explanation for this finding, the high number of follicles punctured suggests that this difference is real and asks the hypothesis of physical and structural changes between the COC of both groups. Indeed, the lipidomic analyses of the oocytes showed changes mostly in phosphoglycerolipid compositions (mostly PC, 12/18 up-regulated lipids and 15/24 down-regulated lipids in n-3 oocytes) and SM, with some lipids being up-regulated and others being down-regulated in n-3 oocytes. PC lipids are a major component of the cell membrane, and SM are involved in membrane structure stabilisation (Zheng et al., 2006), therefore,

these changes could potentially be linked to cell membrane composition, and could be also linked to increased elasticity or suction resistance, allowing for an increased COC recovered rate (observed in n-3 group). Such changes in membrane composition were previously reported (Calder, 2015). Moreover, less TG (mainly involved in energy storage) was reported in n-3 oocytes, which is consistent with previously reported results suggesting that n-3 supplementation leads to increased lipolysis (Elis et al., 2016a). Further studies are necessary to investigate the relationship between the specific lipids that present a change in their composition and an enhanced oocyte quality.

Declaration of interest, Funding and Acknowledgements

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the reported research.

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539 **Figure legends**

540

541 **Figure 1: Experimental design** – Thirty seven cows underwent five ovum pick-up (OPU)
542 sessions to either collect oocyte-cumulus complexes and perform *in vitro* embryo production
543 (OPU sessions 1, 3 and 4), or to collect oocytes and perform lipid composition mass
544 spectrometry analysis (OPU sessions 2 and 5). Cows received a diet supplemented with either
545 PUFA n-3 or PUFA n-6 supplementation during 9 weeks, beginning (=d0, d stands for day) on
546 average 11 weeks postpartum. Each cow received a synchronisation treatment, eleven days
547 prior to d0, consisting of a 3.3 mg norgestomet subcutaneous implant and an intra muscular
548 (IM) injection of 0.001 mg buserelin (Crestar Pack®, MSD Santé Animale, Beaucouzé,
549 France). Seven days later (d-4), each cow received an IM injection of 15 mg luprostriol
550 (Prosolvin®, Virbac, Carros, France) and 2 days later (d-2), the subcutaneous implant was
551 removed. Oestrus was observed ≈48 h following implant removal (d=0), and a new
552 subcutaneous implant of 3.3 mg norgestomet was inserted on the same day (Crestar® SO, MSD
553 Santé Animale, Beaucouzé, France). The norgestomet implant was then replaced every 9 or 10
554 days until the end of the experiment (d64, last day of supplementation), to prevent the
555 occurrence of oestrus between OPU sessions. A FSH treatment (ovarian stimulation with
556 Stimufol® (Reprobiol, Ouffet, Belgium)was performed before OPU 1, OPU 3, OPU 4 and
557 OPU 5.

558

559 **Figure 2: Physiological measurements** – Body weight (A), milk yield (B), dry matter intake
560 (C) and energy balance (D) were monitored in 37 primiparous Holstein cows (n-3, $n = 18$; n-6,
561 $n = 19$). Cows received either a PUFA n-3 diet (black line) or a PUFA n-6 diet (gray line) for 9
562 weeks. Results are presented as lsmeans ± SEM. * indicates a difference ($p < 0.05$); # indicates
563 a tendency ($p < 0.10$).

564

565 **Figure 3: Metabolic measurements** – Plasma NEFA (A), glucose (B) and urea (C) levels were
566 monitored once per week in 37 primiparous Holstein cows (n-3, *n* = 18; n-6, *n* = 19). Cows
567 received either a PUFA n-3 diet (black line) or a PUFA n-6 diet (gray line) for 9 weeks. Results
568 are presented as lsmeans ± SEM. * indicates a difference ($p < 0.05$); # indicates a tendency (p
569 < 0.10).

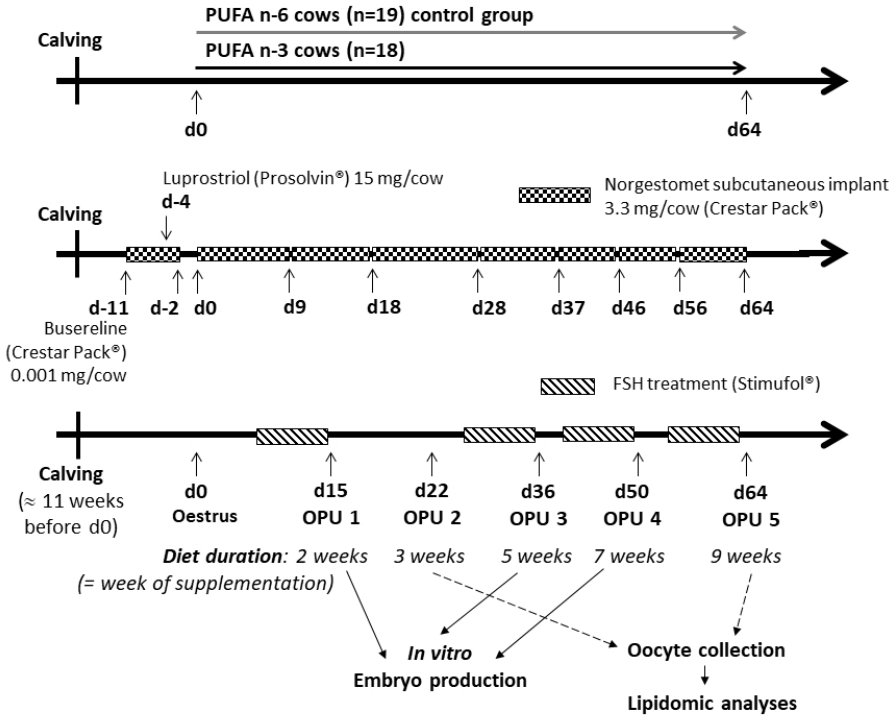
570

571 **Figure 4: Oocyte lipid composition** – 60 oocytes and 61 oocytes, originating from 9 n-3 cows
572 and 9 n-6 cows, respectively, underwent individual lipid analysis by MALDI-TOF mass
573 spectrometry. (A) Hierarchical clustering representing the 110 m/z that exhibited a differential
574 abundance between n-3 and n-6 oocytes (higher abundance represented in green and lower
575 abundance in red). (B) Principle component analysis performed on n-3 and n-6 oocytes. (C)
576 Examples of identified lipids that were either up-regulated or down-regulated in n-3 oocytes.
577 LPC: lyso-phosphatidylcholine; SM: sphingomyelins; PC: phosphatidylcholines; PE:
578 phosphatidylethanolamines; TG: triacylglycerols.

579

580 **Supplementary Figure 1: Physiological and metabolic measurements** - Body weight, milk
581 yield, dry matter intake, energy balance (A) and plasma NEFA, glucose and urea levels (B)
582 were monitored during 9 weeks in 18 (from the 37) primiparous Holstein cows (n-3, *n* = 9; n-
583 6, *n* = 9) providing the oocytes analysed by mass spectrometry, for their lipid composition.
584 Cows received either a PUFA n-3 diet (black line) or a PUFA n-6 diet (gray line) for 9 weeks
585 of supplementation. Linear mixed models (MIXED procedure) included the effects of diet,
586 week of supplementation, diet x week of supplementation interaction and week of
587 supplementation as a repeated effect within cow (repeated statement of the MIXED procedure,
588 an AR(1) covariance structure was used for all parameters, except for plasma and follicular

589 fluid FA composition for which a CS covariance structure was used) were used for the
590 following parameters: BW (with BW at 1 week of supplementation as a covariate), MY (with
591 the day in milk corresponding to the first day of supplementation as a covariate), DMI (with
592 BW as a covariate), EB, plasma NEFA, glucose, urea (with, for each of these three parameters,
593 the plasma level measured the first day of supplementation as a covariate). Results are presented
594 as lsmeans ± SEM. * indicates a difference ($p < 0.05$); # indicates a tendency ($p < 0.10$). No
595 diet effect was found for any measured parameter: BW ($p = 0.5765$), MY ($p = 0.9535$), DMI (p
596 $= 0.6089$), EB ($p = 0.5946$), NEFA ($p = 0.6206$), glucose ($p = 0.4728$) and urea ($p = 0.4339$).
597 A significant week of supplementation effect was found for all parameters, BW ($p < 0.0001$),
598 MY ($p < 0.0001$), DMI ($p = 0.0036$), NEFA ($p < 0.0001$) and glucose ($p = 0.0004$), except for
599 EB ($p = 0.2861$) and urea ($p = 0.9173$). No interaction between diet and week of
600 supplementation was reported for BW ($p = 0.4493$), MY ($p = 0.4474$), DMI ($p = 0.6284$), EB
601 ($p = 0.4318$), NEFA ($p = 0.0951$) and glucose ($p = 0.9527$). A significant interaction between
602 diet and week of supplementation was only reported for urea ($p = 0.0004$).
603



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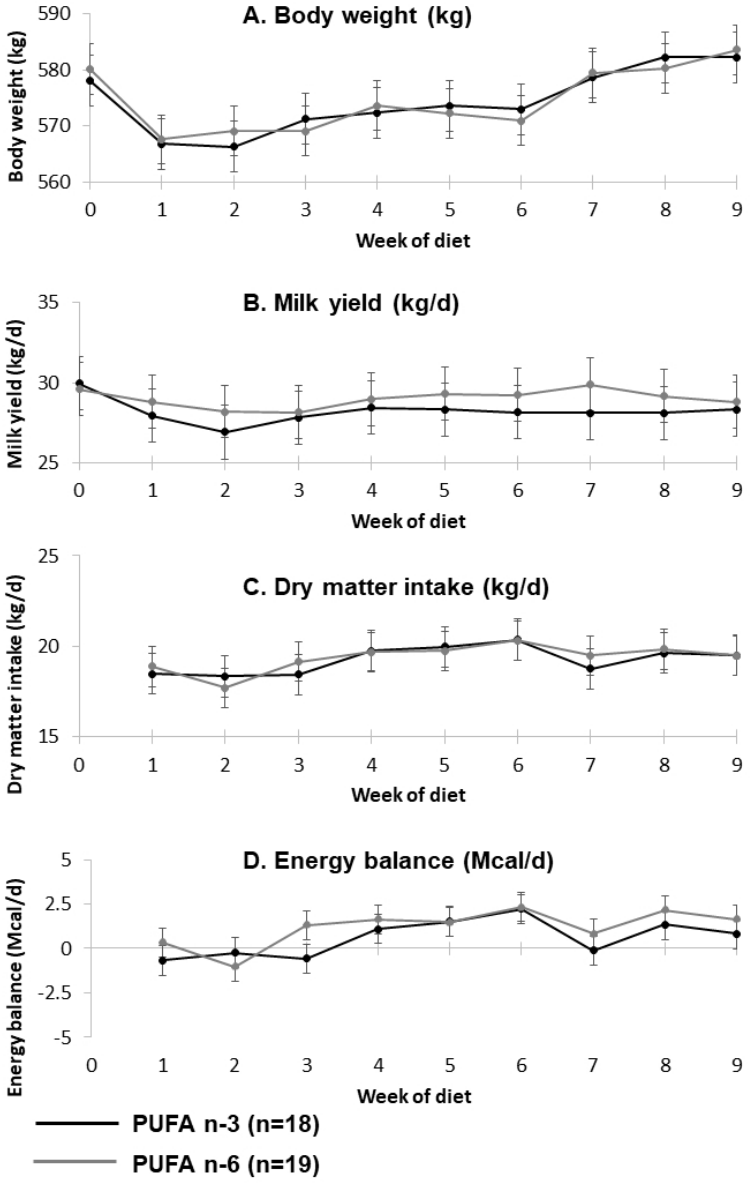

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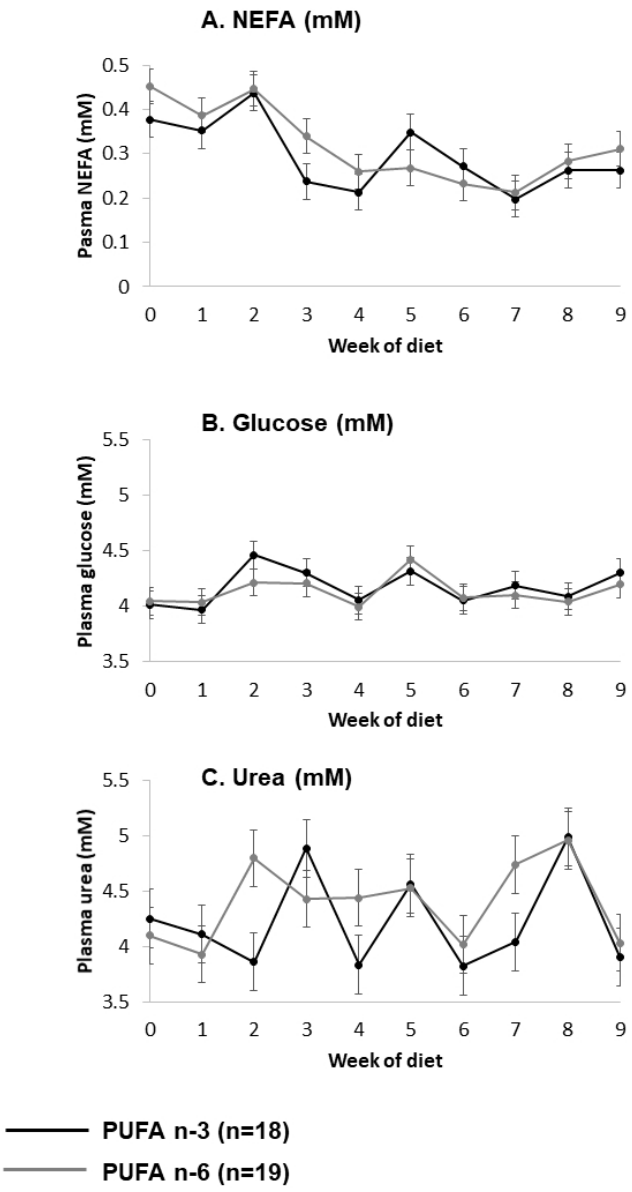

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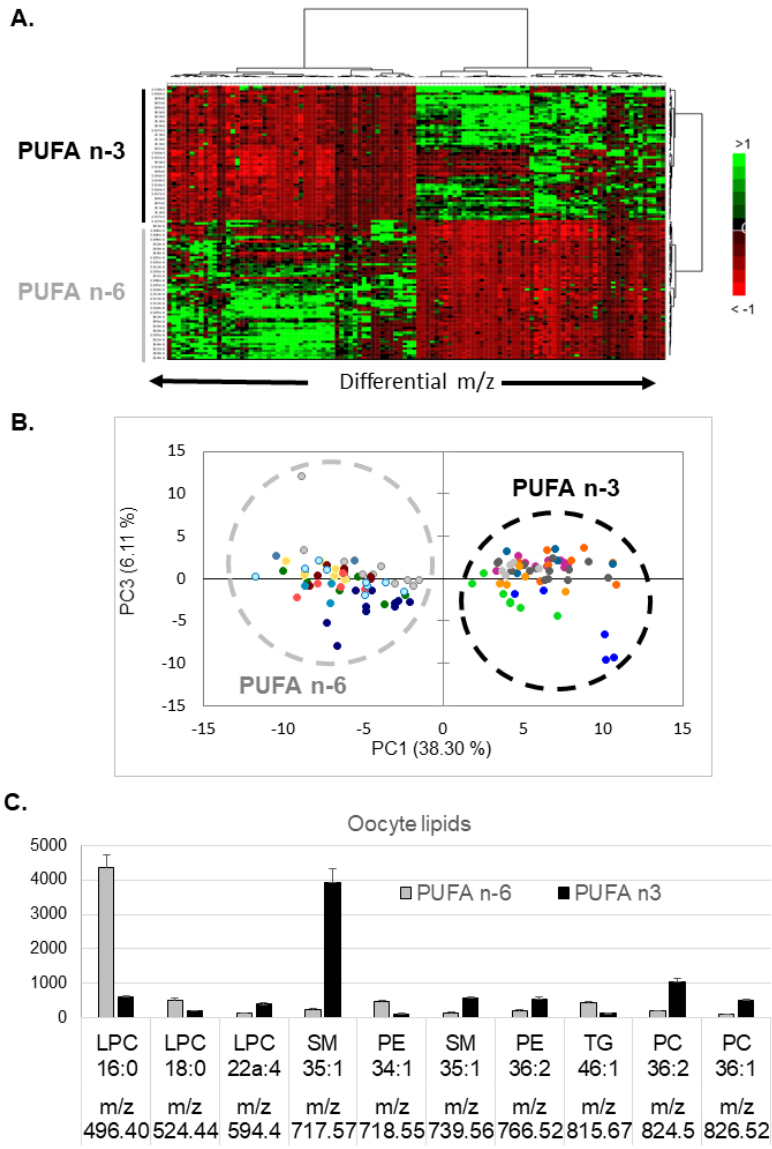


Figure 4: Oocyte lipid composition – 60 oocytes and 61 oocytes, originating from 9 n-3 cows and 9 n-6 cows, respectively, underwent individual lipid analysis by MALDI-TOF mass spectrometry. (A) Hierarchical clustering representing the 110 m/z that exhibited a differential abundance between n-3 and n-6 oocytes (higher abundance represented in green and lower abundance in red). (B) Principle component analysis performed on n-3 and n-6 oocytes. (C) Examples of identified lipids that were either up-regulated or down-regulated in n-3 oocytes. LPC: lyso-phosphatidylcholine; SM: sphingomyelins; PC: phosphatidylcholines; PE: phosphatidylethanolamines; TG: triacylglycerols.

190x275mm (96 x 96 DPI)

Table 1: Effect of a PUFA n-3 (n = 18) or PUFA n-6 diet (n = 19) on plasma fatty acid (FA) composition (% of total fatty acid), expressed as lsmeans ± SEM

	Before diet		2 weeks of diet		5 weeks of diet		7 weeks of diet		Diet effect	Week of suppl° effect	Diet x week of suppl° interaction
	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	p-value	p-value	p-value
C14:0	0.74 ± 0.20	0.72 ± 0.20	0.72 ± 0.20 #	0.65 ± 0.20	0.76 ± 0.20 *	0.66 ± 0.20	0.76 ± 0.20 *	0.64 ± 0.20	**0.03**	0.166	0.1685
C15:0	0.86 ± 0.04	0.81 ± 0.03	0.81 ± 0.04 #	0.75 ± 0.03	0.90 ± 0.04 *	0.79 ± 0.03	0.91 ± 0.04 *	0.80 ± 0.03	**0.0016**	0.0008	0.3320
C16:0	16.2 ± 1.5	16.7 ± 1.5	18.5 ± 1.5 #	17.3 ± 1.5	16.4 ± 1.5	15.4 ± 1.5	16.4 ± 1.5	15.5 ± 1.5	0.1324	<0.0001	0.242
C18:0	19.4 ± 1.8	19.9 ± 1.8	18.7 ± 1.8	18.9 ± 1.8	18.4 ± 1.8	18.2 ± 1.8	18.7 ± 1.8	18.7 ± 1.8	0.8535	0.0036	0.7891
C20:0	0.35 ± 0.08	0.35 ± 0.08	0.43 ± 0.08	0.38 ± 0.08	0.49 ± 0.08 *	0.31 ± 0.08	0.32 ± 0.08	0.39 ± 0.08	0.5134	0.6393	0.098
C22:0	0.10 ± 0.07	0.05 ± 0.07	0.03 ± 0.07	0.11 ± 0.07	0.10 ± 0.07	0.10 ± 0.07	0.01 ± 0.07	0.07 ± 0.07	0.54	0.4166	0.256
C24:0	0.35 ± 0.04	0.37 ± 0.03	0.45 ± 0.04 *	0.35 ± 0.03	0.35 ± 0.04	0.34 ± 0.03	0.44 ± 0.04 #	0.37 ± 0.03	**0.0556**	0.0826	0.1621
SFA	37.7 ± 3.2	38.6 ± 3.2	39.2 ± 3.2	38.1 ± 3.2	37.0 ± 3.2	35.4 ± 3.2	37.3 ± 3.2	36.2 ± 3.2	0.3840	0.0041	0.3867
C14:1	0.52 ± 0.04 *	0.39 ± 0.04	0.43 ± 0.04	0.38 ± 0.04	0.49 ± 0.04 *	0.36 ± 0.04	0.50 ± 0.04 *	0.40 ± 0.04	**0.0013**	0.0826	0.3261
C16:1	1.54 ± 0.20	1.59 ± 0.19	1.42 ± 0.20	1.36 ± 0.19	1.40 ± 0.20 *	1.06 ± 0.19	1.33 ± 0.20 *	0.91 ± 0.19	**0.0389**	<0.0001	0.0027
C18:1	14.5 ± 0.9	14.9 ± 0.9	14.1 ± 0.9	14.0 ± 0.9	12.0 ± 0.9	11.3 ± 0.9	11.9 ± 0.9 *	10.4 ± 0.9	0.2282	<0.0001	0.1769
C20:1 n-9	0.10 ± 0.09	0.12 ± 0.08	0.10 ± 0.09	0.17 ± 0.08	0.07 ± 0.09 #	0.29 ± 0.08	0.17 ± 0.09	0.18 ± 0.08	0.1191	0.8054	0.6134
C22:1 n-9	0.26 ± 0.14	0.39 ± 0.13	0.40 ± 0.14	0.48 ± 0.13	0.33 ± 0.14	0.30 ± 0.13	0.50 ± 0.14	0.30 ± 0.13	0.9625	0.6226	0.4646
C24:1 n-9	0.24 ± 0.04	0.24 ± 0.04	0.25 ± 0.04	0.20 ± 0.04	0.20 ± 0.04	0.19 ± 0.04	0.24 ± 0.04	0.20 ± 0.04	0.3278	0.4629	0.6383
MUFA	16.9 ± 0.9	17.4 ± 0.9	16.4 ± 0.9	16.2 ± 0.9	14.2 ± 0.9	13.2 ± 0.9 *	14.3 ± 0.9	12.1 ± 0.9	0.1022	<0.0001	0.0215
C18:2 n-6	35.8 ± 3.2	34.3 ± 3.1	34.8 ± 3.2	36.9 ± 3.1	37.9 ± 3.2 *	42.2 ± 3.1	38.4 ± 3.2 *	42.7 ± 3.1	**0.0161**	<0.0001	0.0022
C18:3 n-6	0.80 ± 0.11 *	0.65 ± 0.11	0.47 ± 0.11	0.52 ± 0.11	0.73 ± 0.11 *	0.52 ± 0.11	0.59 ± 0.11	0.59 ± 0.11	0.1955	<0.0001	0.0023
C20:3 n-6	1.64 ± 0.11	1.77 ± 0.10	1.33 ± 0.11	1.49 ± 0.10	1.31 ± 0.11 *	1.60 ± 0.10	1.31 ± 0.11 *	1.61 ± 0.10	**0.0076**	<0.0001	0.4576
C20:4 n-6	1.44 ± 0.26	1.16 ± 0.26	1.05 ± 0.26	1.20 ± 0.26	1.27 ± 0.26	1.34 ± 0.26	1.00 ± 0.26	1.29 ± 0.26	0.6918	0.1844	0.0597
n-6	39.7 ± 3.5	37.9 ± 3.5	37.6 ± 3.5 #	40.1 ± 3.5	41.5 ± 3.5 *	45.7 ± 3.5	41.3 ± 3.5 *	46.2 ± 3.5	**0.0081**	<0.0001	0.0012
C18:3 n-3	4.09 ± 0.40	4.25 ± 0.40	4.30 ± 0.40 #	3.85 ± 0.40	4.51 ± 0.40 #	4.06 ± 0.40	4.45 ± 0.40 *	3.89 ± 0.40	**0.0340**	0.6004	0.0892
C20:5 n-3	**0.72 ± 0.15**	**0.84 ± 0.15**	**1.45 ± 0.15 ***	**0.85 ± 0.15**	**1.60 ± 0.15 ***	**0.82 ± 0.15**	**1.63 ± 0.15 ***	**0.78 ± 0.15**	**<0.0001**	<0.0001	<0.0001
C22:5 n-3	0.74 ± 0.06	0.81 ± 0.06	0.78 ± 0.06	0.72 ± 0.06	0.85 ± 0.06 *	0.65 ± 0.06	0.81 ± 0.06 *	0.62 ± 0.06	**0.0010**	0.2900	0.0002
C22:6 n-3	**0.11 ± 0.04**	**0.18 ± 0.04**	**0.23 ± 0.04**	**0.22 ± 0.04**	**0.32 ± 0.04 #**	**0.21 ± 0.04**	**0.34 ± 0.04 ***	**0.21 ± 0.04**	0.4164	<0.0001	<0.0001
n-3	5.7 ± 0.6	6.1 ± 0.6	6.8 ± 0.6 *	5.6 ± 0.6	7.3 ± 0.6 *	5.7 ± 0.6	7.2 ± 0.6 *	5.5 ± 0.6	**<0.0001**	0.0033	<0.0001
PUFA	45.4 ± 4.0	44.0 ± 4.0	44.4 ± 4.0	45.8 ± 4.0	48.8 ± 4.0 #	51.5 ± 4.0	48.5 ± 4.0 *	51.8 ± 4.0	0.1224	<0.0001	0.0991
n-6 / n-3	7.7 ± 0.4 *	6.3 ± 0.4	5.5 ± 0.4 *	7.2 ± 0.4	5.9 ± 0.5 *	8.2 ± 0.4	6.2 ± 0.4 *	8.6 ± 0.4	**0.0010**	0.0611	<0.0001

n-3: n-3 polyunsaturated fatty acids; n-6: n-6 polyunsaturated fatty acids; week of suppl°: week of supplementation; SFA = sum of saturated FA; MUFA = sum of monounsaturated FA; PUFA = sum of polyunsaturated FA; n-3 = sum of n-3 FA; n-6 = sum of n-6 FA; C20:5 n-3: eicosapentaenoic acid (EPA); C22:6 n-3: docosahexaenoic acid (DHA); # indicates a tendency ($p < 0.1$) within a row and a stage * indicates a significant difference between diets ($p <$

0.05). The sample size was 18 n-3 and 19 n-6 cows, except for C20:0, C22:0, C24:0, C22:1 n-9, C24:1 n-9 (11 n-3 and 13 n-6 cows) and C20:1 n-9 (8 n-3 and 11 n-6 cows).

Table 2: Effect of a PUFA n-3 (n = 18) or PUFA n-6 diet (n = 19) on follicular fluid fatty acid composition (% of total fatty acid), expressed as lsmeans ± SEM

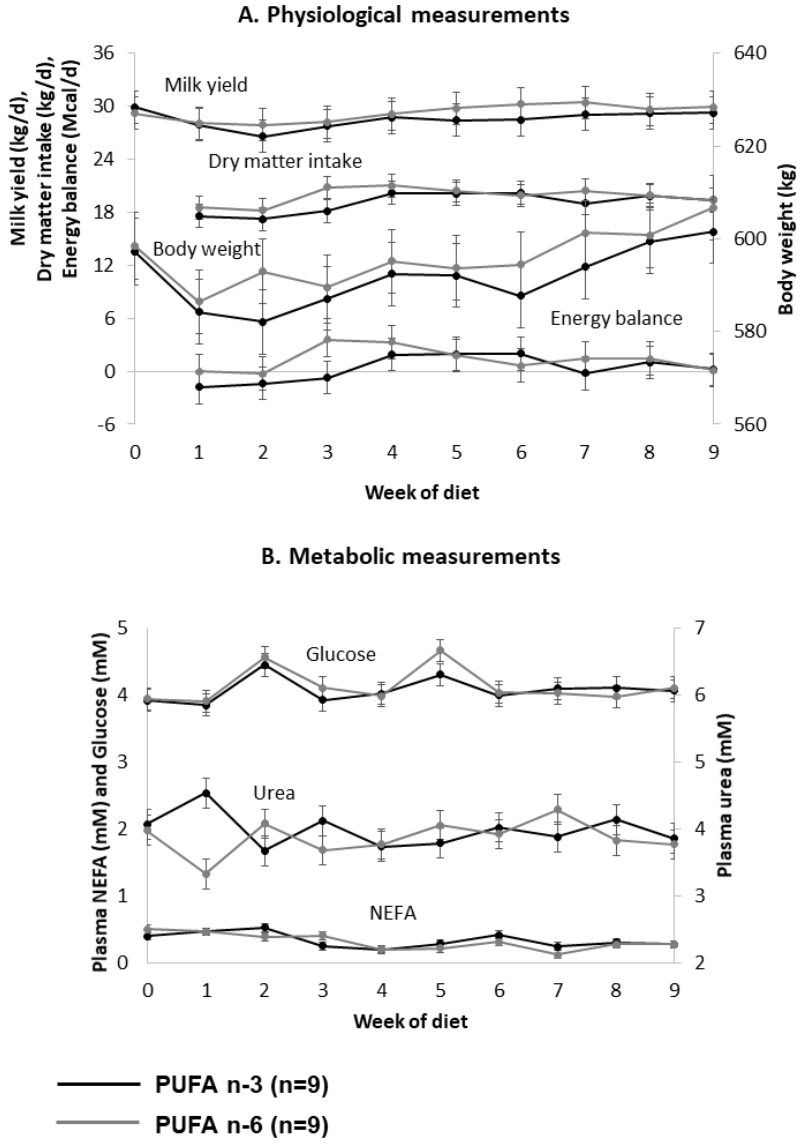
	2 weeks of diet		5 weeks of diet		7 weeks of diet		Diet effect	Week of suppl° effect	Diet x week of suppl° interaction
	n-3	n-6	n-3	n-6	n-3	n-6	p-value	p-value	p-value
C14:0	0.88 ± 0.17	0.84 ± 0.17	0.98 ± 0.17	0.90 ± 0.17	1.02 ± 0.17	1.02 ± 0.17	0.6037	0.0046	0.7281
C15:0	0.75 ± 0.06	0.73 ± 0.05	0.78 ± 0.06	0.76 ± 0.05	0.84 ± 0.06	0.81 ± 0.06	0.6625	0.0324	0.9767
C16:0	20.4 ± 3.2	19.6 ± 3.2	18.9 ± 3.2	20.0 ± 3.2	19.6 ± 3.2	21.1 ± 3.2	0.6534	0.5145	0.2928
C18:0	20.3 ± 4.2	19.6 ± 4.2	19.8 ± 4.2	20.8 ± 4.2	19.3 ± 4.2	20.0 ± 4.2	0.7860	0.7052	0.5502
C20:0	0.90 ± 0.30	0.66 ± 0.30	0.72 ± 0.30	0.37 ± 0.30	0.67 ± 0.30	0.71 ± 0.30	0.31	0.3173	0.4216
C22:0	0.74 ± 0.15	0.62 ± 0.14	0.72 ± 0.15	0.65 ± 0.14	0.82 ± 0.15 #	0.61 ± 0.14	0.0824	0.8147	0.5884
C24:0	0.75 ± 0.14	0.60 ± 0.14	0.75 ± 0.14	0.73 ± 0.14	0.81 ± 0.14	0.74 ± 0.14	0.3009	0.3795	0.6699
SFA	43.7 ± 7.8	41.8 ± 7.8	41.8 ± 7.8	43.5 ± 7.8	42.2 ± 7.8	44.1 ± 7.8	0.8058	0.9311	0.2788
C14:1	0.45 ± 0.09 *	0.37 ± 0.09	0.47 ± 0.09 *	0.36 ± 0.09	0.47 ± 0.09 *	0.38 ± 0.09	**0.0043**	0.7765	0.8179
C16:1	1.29 ± 0.31	1.33 ± 0.31	1.33 ± 0.31	1.08 ± 0.31	1.29 ± 0.31	1.03 ± 0.31	0.2962	0.2950	0.2139
C18:1	14.8 ± 1.6	14.8 ± 1.6	12.9 ± 1.6	11.3 ± 1.6	11.7 ± 1.6	11.0 ± 1.6	0.3971	<0.0001	0.2170
C20:1 n-9	0.12 ± 0.13	0.25 ± 0.13	0.21 ± 0.13	0.14 ± 0.13	0.12 ± 0.13	0.33 ± 0.12	0.4003	0.8346	0.2662
C22:1 n-9	0.04 ± 0.06 #	0.16 ± 0.06	0.12 ± 0.06	0.14 ± 0.06	0.19 ± 0.06 #	0.04 ± 0.06	0.9061	0.8434	0.0119
C24:1 n-9	0.40 ± 0.05	0.35 ± 0.05	0.38 ± 0.05	0.32 ± 0.05	0.41 ± 0.05	0.32 ± 0.05	0.2105	0.8662	0.8527
MUFA	16.9 ± 1.5	16.9 ± 1.5	15.1 ± 1.5 #	13.1 ± 1.5	13.9 ± 1.5	12.8 ± 1.5	0.2839	<0.0001	0.0713
C18:2 n-6	30.0 ± 6.6	32.5 ± 6.6	33.1 ± 6.6	34.9 ± 6.6	33.8 ± 6.6	34.8 ± 6.6	0.2737	0.0081	0.7997
C18:3 n-6	0.38 ± 0.10	0.39 ± 0.10	0.44 ± 0.10	0.42 ± 0.10	0.53 ± 0.10	0.49 ± 0.10	0.7028	0.0790	0.8812
C20:3 n-6	1.51 ± 0.11	1.59 ± 0.11	1.29 ± 0.11	1.35 ± 0.11	1.40 ± 0.11	1.47 ± 0.11	0.4929	0.0936	0.9991
C20:4 n-6	1.84 ± 0.22	1.66 ± 0.22	1.60 ± 0.22	1.56 ± 0.22	1.57 ± 0.22	1.35 ± 0.22	**0.0792**	0.0449	0.7231
n-6	33.7 ± 6.9	36.1 ± 6.9	36.4 ± 6.9	38.3 ± 6.9	37.3 ± 6.9	38.1 ± 6.9	0.3183	0.0128	0.7415
C18:3 n-3	3.52 ± 0.82	3.31 ± 0.81	4.02 ± 0.82 *	3.37 ± 0.81	3.97 ± 0.82 *	3.19 ± 0.82	**0.0038**	0.1401	0.1113
C20:5 n-3	**1.06 ± 0.22** *	**0.81 ± 0.22**	**1.41 ± 0.22** *	**0.77 ± 0.27**	**1.51 ± 0.22** *	**0.77 ± 0.22**	**<0.0001**	0.0033	0.0002
C22:5 n-3	0.85 ± 0.07	0.82 ± 0.07	0.86 ± 0.07 #	0.72 ± 0.07	0.84 ± 0.07 #	0.71 ± 0.07	0.0742	0.4225	0.4006
C22:6 n-3	**0.22 ± 0.10**	**0.19 ± 0.10**	**0.32 ± 0.10**	**0.24 ± 0.10**	**0.32 ± 0.10**	**0.22 ± 0.10**	0.1863	0.0437	0.5507
n-3	5.7 ± 1.1	5.1 ± 1.1	6.6 ± 1.1 *	5.1 ± 1.1	6.6 ± 1.1 *	4.8 ± 1.1	**<0.0001**	0.0557	0.0021
PUFA	39.4 ± 7.9	41.2 ± 7.9	43.0± 7.9	43.3 ± 7.9	44.0 ± 7.9	43.0 ± 7.9	0.8310	0.0117	0.4684
ratio n-6 / n-3	5.9 ± 1.0	7.2 ± 1.0	5.6 ± 1.0	7.6 ± 1.0	6.5 ± 1.0 *	10.3 ± 1.0	**0.0003**	0.1178	0.4511

n-3: n-3 polyunsaturated fatty acids; n-6: n-6 polyunsaturated fatty acids; week of suppl°: week of supplementation; SFA = sum of saturated FA; MUFA = sum of monounsaturated FA; PUFA = sum of polyunsaturated FA; n-3 = sum of n-3 FA; and n-6 = sum of n-6 FA; C20:5 n-3: eicosapentaenoic acid (EPA); C22:6 n-3: docosahexaenoic acid (DHA); # indicates a tendency ($p < 0.1$) within a row and a stage * indicates a significant difference between diets ($p < 0.05$). The sample size was 18 n-3 and 19 n-6 cows, except for C20:0, C22:0, C24:0, C22:1 n-9, C24:1 n-9 (11 n-3 and 13 n-6 cows) and C20:1 n-9 (8 n-3 and 9 n-6 cows).

Table 3: Effect of a PUFA n-3 (n = 18) or PUFA n-6 diet (n = 19) on rates (%) and mean numbers per OPU-IVF session (Nb) for COC collection and embryo production, expressed as lsmeans ± SEM

IVM/IVF/IVD	n-3	n-6	2 weeks of diet (OPU session 1)		5 weeks of diet (OPU session 3)		7 weeks of diet (OPU session 4)		Diet effect	Week of suppl° effect	Diet x week of suppl° interaction
			n-3	n-6	n-3	n-6	n-3	n-6	p-value	p-value	p-value
% Recovered COC	38.0±1.6 *	32.8±1.6	42.0±2.5 #	36.5±2.5	38.4±2.4 *	31.2±2.2	33.7±2.4	30.8±2.2	**0.0035**	**0.0054**	0.6198
% Oocytes in IVM	76.1±2.5	73.0±2.8	73.3±3.6	70.9±4.1	79.0±3.4 *	68.6±4.2	75.7±3.9	78.7±3.6	0.2586	0.3138	0.144
% Cleaved embryos	77.3±3.8 #	82.3±3.3	76.6±4.8	84.7±4.2	71.3±5.2	79.3±5.0	83.0±4.4	82.7±4.3	**0.1033**	0.1268	0.4831
% Embryos >4-cell	53.6±4.1	55.8±4.2	50.7±5.3	54.6±5.8	50.8±5.2	54.1±5.8	59.3±5.7	54.3±5.4	0.5649	0.2802	0.8622
% Blastocysts	49.6±5.5 #	42.3±5.5	48.5±6.7 #	37.0±6.6	52.3±6.8	43.2±7.2	47.8±7.0	46.9±6.8	**0.0865**	0.5439	0.528
% Blastocysts Q1-Q2	42.2±7.7 *	32.7±7.1	42.2±8.6 #	30.0±7.8	43.7±8.8 *	29.3±7.9	40.7±8.9	39.2±8.6	**0.0217**	0.6595	0.3499
Nb Punctured follicles	26.7±4.3	26.1±4.3	26.5±4.5	22.5±4.4	28.1±4.5	27.5±4.4	25.7±4.5	28.3±4.4	0.8196	**0.0674**	0.1868
Nb Recovered COC	10.2±1.3	8.5±1.3	11.1±1.5	8.3±1.5	10.8±1.5	8.6±1.5	8.6±1.5	8.7±1.5	0.2297	0.5125	0.3641
Nb Oocytes in IVM	7.8±1.1	6.2±1.1	8.2±1.3	5.8±1.3	8.6±1.3	5.9±1.3	6.6±1.3	6.8±1.3	0.1729	0.7662	0.166
Nb Cleaved embryos	5.8±1.0	5.0±1.0	6.0±1.1	4.7±1.1	6.1±1.1	4.5±1.1	5.4±1.1	5.7±1.1	0.3597	0.9619	0.4046
Nb Embryos > 4-cell	4.1±0.8	3.4±0.8	4.1±0.9	3.1±0.9	4.4±0.9	3.2±0.9	3.7±0.9	4.1±0.9	0.4297	0.8714	0.29
Nb Blastocysts	2.8±0.6	2.2±0.6	3.0±0.7	1.8±0.7	3.1±0.7	1.9±0.7	2.5±0.7	2.8±0.7	0.2136	0.9005	0.1917
Nb Blastocysts Q1-Q2	2.5±0.5	1.8±0.5	2.6±0.6	1.6±0.6	2.6±0.6	1.3±0.6	2.1±0.6	2.4±0.6	0.1527	0.6997	0.1378

%: rates; Nb: number per cow per OPU-IVF session; OPU: ovum pick-up; COC: complex oocyte-cumulus; IVM: *in vitro* maturation; IVF: *in vitro* fertilisation; IVD: *in vitro* development; week of suppl°: week of supplementation (= rank of OPU session); Blastocysts Q1-Q2: blastocysts of freezable quality according to IETS (International Embryo Technology Society) evaluation criteria; % recovered COC is a ratio to punctured follicles; % Oocytes in IVM is a ratio to recovered COC; rates of cleaved embryos (> 2 cells) and of > 4-cell embryo are ratios to COC in IVM; blastocysts and blastocysts Q1-Q2 are ratios to cleaved embryos. # indicates a tendency ($p < 0.1$) within a row and a stage; * indicates a significant difference between diets ($p < 0.05$). The sample size was 1462 punctured follicles in 18 n-3 cows and 1538 punctured follicles in 19 n-6 cows, corresponding to 54 and 57 OPU sessions, respectively.



Supplementary Figure 1: Physiological and metabolic measurements - Body weight, milk yield, dry matter intake, energy balance (A) and plasma NEFA, glucose and urea levels (B) were monitored during 9 weeks in 18 (from the 37) primiparous Holstein cows (n-3, n = 9; n-6, n = 9) providing the oocytes analysed by mass spectrometry, for their lipid composition. Cows received either a PUFA n-3 diet (black line) or a PUFA n-6 diet (gray line) for 9 weeks of supplementation. Linear mixed models (MIXED procedure) included the effects of diet, week of supplementation, diet x week of supplementation interaction and week of supplementation as a repeated effect within cow (repeated statement of the MIXED procedure, an AR(1) covariance structure was used for all parameters, except for plasma and follicular fluid FA composition for which a CS covariance structure was used) were used for the following parameters: BW (with BW at 1 week of supplementation as a covariate), MY (with the day in milk corresponding to the first day of supplementation as a covariate), DMI (with BW as a covariate), EB, plasma NEFA, glucose, urea (with, for each of these three parameters, the plasma level measured the first day of supplementation as a covariate). Results are presented as lsmeans ± SEM. * indicates a difference ($p < 0.05$); # indicates a tendency ($p < 0.10$). No diet effect was found for any measured parameter: BW ($p = 0.5765$), MY ($p = 0.9535$), DMI ($p =$

0.6089), EB (p = 0.5946), NEFA (p = 0.6206), glucose (p = 0.4728) and urea (p = 0.4339). A significant week of supplementation effect was found for all parameters, BW (p < 0.0001), MY (p < 0.0001), DMI (p = 0.0036), NEFA (p < 0.0001) and glucose (p = 0.0004), except for EB (p = 0.2861) and urea (p = 0.9173). No interaction between diet and week of supplementation was reported for BW (p = 0.4493), MY (p = 0.4474), DMI (p = 0.6284), EB (p = 0.4318), NEFA (p = 0.0951) and glucose (p = 0.9527). A significant interaction between diet and week of supplementation was only reported for urea (p = 0.0004).

190x275mm (96 x 96 DPI)

Supplementary Table 1. Effect of a PUFA n-3 (n = 9) or PUFA n-6 diet (n = 9) on plasma fatty acid composition (% of total fatty acid) of the cows providing oocytes undergoing mass spectrometry lipid analysis, expressed as lsmeans ± SEM

	before diet		2 weeks of diet		5 weeks of diet		7 weeks of diet		diet effect	Week of suppl°	diet x Week of suppl°
	n-3	n-6	n-3	n-6	n-3	n-6	n-3	n-6	p-value	effect	interaction
C14:0	0.38 ± 0.05	0.41 ± 0.05	0.34 ± 0.05	0.33 ± 0.05	0.38 ± 0.05	0.32 ± 0.05	0.40 ± 0.05	0.41 ± 0.05	0.6735	0.5446	0.6589
C15:0	0.88 ± 0.04 #	0.76 ± 0.04	0.76 ± 0.04	0.71 ± 0.04	0.91 ± 0.04 *	0.78 ± 0.04	0.90 ± 0.04 #	0.79 ± 0.04	**0.0468**	0.0047	0.5521
C16:0	17.4 ± 0.9	18.4 ± 0.9	21.5 ± 0.9 *	18.2 ± 0.9	18.1 ± 0.9	16.3 ± 0.9	18.8 ± 0.9 #	16.4 ± 0.9	**0.1112**	0.0018	0.0202
C18:0	21.7 ± 1.0	23.0 ± 1.0	21.7 ± 1.0	22.3 ± 1.0	21.2 ± 1.0	21.0 ± 1.0	22.2 ± 1.0	21.9 ± 1.0	0.7553	0.272	0.5778
C20:0	0.29 ± 0.08	0.31 ± 0.08	0.43 ± 0.08	0.32 ± 0.08	0.48 ± 0.08	0.30 ± 0.08	0.37 ± 0.08	0.38 ± 0.08	0.478	0.4215	0.2613
C22:0	0.12 ± 0.06	0.09 ± 0.06	0.04 ± 0.06	0.18 ± 0.06	0.12 ± 0.06	0.16 ± 0.06	0.02 ± 0.06	0.11 ± 0.06	0.4294	0.4496	0.2807
C24:0	0.36 ± 0.04	0.39 ± 0.04	0.47 ± 0.04	0.37 ± 0.04	0.38 ± 0.04	0.34 ± 0.04	0.47 ± 0.04	0.39 ± 0.04	0.1749	0.2002	0.2862
SFA	41.1 ± 1.6	43.3 ± 1.6	45.3 ± 1.6	42.4 ± 1.6	41.6 ± 1.6	39.2 ± 1.6	43.1 ± 1.6	40.3 ± 1.6	0.4079	0.0588	0.1273
C14:1	0.52 ± 0.05 *	0.27 ± 0.05	0.36 ± 0.05	0.30 ± 0.05	0.47 ± 0.05 *	0.27 ± 0.05	0.46 ± 0.05	0.34 ± 0.05	**0.0033**	0.3695	0.1321
C16:1	1.30 ± 0.14	1.24 ± 0.14	1.07 ± 0.14	1.02 ± 0.14	1.10 ± 0.14	0.68 ± 0.14	0.91 ± 0.14	0.52 ± 0.14	0.1262	0.0002	0.2188
C18:1	15.9 ± 0.8	17.0 ± 0.8	16.3 ± 0.8	15.7 ± 0.8	12.3 ± 0.8	12.0 ± 0.8	12.6 ± 0.8	10.7 ± 0.8	0.5642	< 0.0001	0.2331
C20:1 n-9	0.13 ± 0.11	0.14 ± 0.10	0.08 ± 0.11	0.26 ± 0.10	0.08 ± 0.11 #	0.37 ± 0.10	0.05 ± 0.11	0.16 ± 0.10	**0.0194**	0.7247	0.6541
C22:1 n-9	0.28 ± 0.15	0.23 ± 0.15	0.46 ± 0.15	0.42 ± 0.15	0.28 ± 0.15	0.32 ± 0.15	0.51 ± 0.15	0.30 ± 0.15	0.664	0.4634	0.7989
C24:1 n-9	0.26 ± 0.04	0.28 ± 0.04	0.26 ± 0.04	0.23 ± 0.04	0.22 ± 0.04	0.20 ± 0.04	0.24 ± 0.04	0.24 ± 0.04	0.8735	0.4628	0.9042
MUFA	18.4 ± 0.8	19.2 ± 0.8	18.5 ± 0.8	17.9 ± 0.8	14.4 ± 0.8	13.8 ± 0.8	14.9 ± 0.8	12.3 ± 0.8	0.3869	< 0.0001	0.1212
C18:2 n-6	32.3 ± 1.8	29.5 ± 1.8	28.7 ± 1.8	32.5 ± 1.8	34.3 ± 1.8	39.2 ± 1.8	34.1 ± 1.8	39.7 ± 1.8	0.1727	< 0.0001	0.0111
C18:3 n-6	0.66 ± 0.10	0.42 ± 0.10	0.29 ± 0.10	0.34 ± 0.10	0.72 ± 0.10	0.33 ± 0.10	0.50 ± 0.10	0.41 ± 0.10	0.1622	0.0043	0.0087
C20:3 n-6	1.42 ± 0.15	1.78 ± 0.15	1.24 ± 0.15	1.34 ± 0.15	1.36 ± 0.15	1.63 ± 0.15	1.36 ± 0.15	1.59 ± 0.15	0.146	0.0732	0.7251
C20:4 n-6	1.17 ± 0.27	0.68 ± 0.27	0.28 ± 0.27	0.79 ± 0.27	0.99 ± 0.27	1.02 ± 0.27	0.46 ± 0.27	0.96 ± 0.27	0.6424	0.0936	0.0511
n-6	35.6 ± 1.8	32.4 ± 1.8	30.5 ± 1.8	35.0 ± 1.8	37.8 ± 1.8	42.2 ± 1.8	36.5 ± 1.8	42.7 ± 1.8	0.1345	< 0.0001	0.0049
C18:3 n-3	3.54 ± 0.30	3.53 ± 0.30	3.56 ± 0.30	3.21 ± 0.30	3.93 ± 0.30	3.48 ± 0.30	3.44 ± 0.30	3.31 ± 0.30	0.3286	0.6293	0.8653
C20:5 n-3	0.61 ± 0.09	0.61 ± 0.09	1.22 ± 0.09 *	0.63 ± 0.09	1.19 ± 0.09 *	0.59 ± 0.09	1.22 ± 0.09 *	0.59 ± 0.09	**0.0002**	< 0.0001	< 0.0001
C22:5 n-3	0.63 ± 0.05 *	0.78 ± 0.05	0.73 ± 0.05	0.62 ± 0.05	0.81 ± 0.05 *	0.52 ± 0.05	0.73 ± 0.05 *	0.59 ± 0.05	**0.0331**	0.7867	0.0004
C22:6 n-3	0.08 ± 0.08	0.23 ± 0.08	0.18 ± 0.08	0.28 ± 0.08	0.22 ± 0.08	0.24 ± 0.08	0.27 ± 0.08	0.26 ± 0.08	0.55	< 0.0001	0.0005
n-3	4.9 ± 0.3	5.2 ± 0.3	5.7 ± 0.3 *	4.7 ± 0.3	6.2 ± 0.3 *	4.8 ± 0.3	5.7 ± 0.3 #	4.7 ± 0.3	**0.0196**	0.4726	0.0599
PUFA	40.5 ± 1.9	37.5 ± 1.9	36.2 ± 1.9	39.8 ± 1.9	43.9 ± 1.9	47.0 ± 1.9	42.1 ± 1.9	47.4 ± 1.9	0.2614	< 0.0001	0.0557
ratio 16:0 / 16:1	14.0 ± 3.9	18.3 ± 3.9	23.2 ± 3.9	21.3 ± 3.9	18.8 ± 3.9	27.3 ± 3.9	28.7 ± 3.9	37.9 ± 3.9	0.1498	0.0003	0.4093

ratio 18:0 / 18:1	1.38 ± 0.14	1.39 ± 0.14	1.35 ± 0.14	1.46 ± 0.14	1.76 ± 0.14	1.80 ± 0.14	1.80 ± 0.14	2.17 ± 0.14	0.4156	< 0.0001	0.1625
ratio n-6 / n-3	8.7 ± 0.8	6.3 ± 0.8	5.3 ± 0.8	7.4 ± 0.8	6.3 ± 0.8	8.9 ± 0.8	7.0 ± 0.8	9.2 ± 0.8	0.1178	0.1735	0.0074

n-3: n-3 polyunsaturated fatty acids; n-6: n-6 polyunsaturated fatty acids; week of suppl°: week of supplementation; SFA = sum of saturated FA; MUFA = sum of monounsaturated FA; PUFA = sum of polyunsaturated FA; n-3= sum of n-3 FA; and n-6 = sum of n-6 FA; C20:5 n-3: eicosapentaenoic acid (EPA); C22:6 n-3: docosahexaenoic acid (DHA); # indicates within a row and a stage a tendency ($p < 0.1$) and * indicates a significant difference between both diets ($p < 0.05$).

Supplementary Table 2. Effect of a PUFA n-3 (n = 9) or PUFA n-6 diet (n = 9) on follicular fluid fatty acid composition (% of total fatty acid) of the cows providing oocytes undergoing mass spectrometry lipid analysis, expressed as lsmeans ± SEM

	2 weeks of diet		5 weeks of diet		7 weeks of diet		diet effect	Week of suppl°	diet x Week of suppl°
	n-3	n-6	n-3	n-6	n-3	n-6	p-value	effect	interaction
C14:0	0.62 ± 0.11	0.72 ± 0.11	0.71 ± 0.11	0.80 ± 0.11	0.69 ± 0.11	0.97 ± 0.11	0.2046	0.1999	0.4647
C15:0	0.77 ± 0.09	0.78 ± 0.09	0.77 ± 0.09	0.86 ± 0.09	0.84 ± 0.09	0.97 ± 0.09	0.459	0.1254	0.6847
C16:0	25.3 ± 2.4	25.4 ± 2.4	23.7 ± 2.4	27.8 ± 2.4	25.4 ± 2.4	29.5 ± 2.4	0.3632	0.3354	0.3029
C18:0	27.8 ± 2.6	26.3 ± 2.6	26.6 ± 2.6	30.9 ± 2.6	27.3 ± 2.6	29.3 ± 2.6	0.6497	0.3939	0.0909
C20:0	1.07 ± 0.24	0.82 ± 0.24	0.87 ± 0.24	0.49 ± 0.24	0.80 ± 0.24	0.71 ± 0.24	0.4106	0.3053	0.7127
C22:0	0.77 ± 0.09	0.76 ± 0.09	0.78 ± 0.09	0.77 ± 0.09	0.92 ± 0.09	0.81 ± 0.09	0.6396	0.3217	0.7494
C24:0	0.78 ± 0.11	0.72 ± 0.11	0.83 ± 0.11	0.88 ± 0.11	0.88 ± 0.11	0.87 ± 0.11	0.948	0.3859	0.8698
SFA	57.1 ± 4.8	55.4 ± 4.8	54.3 ± 4.8	62.5 ± 4.8	56.8 ± 4.8	63.1 ± 4.8	0.5065	0.2766	0.0847
C14:1	0.31 ± 0.05	0.27 ± 0.05	0.33 ± 0.05	0.26 ± 0.05	0.33 ± 0.05	0.29 ± 0.05	0.3419	0.8278	0.8771
C16:1	0.64 ± 0.21	0.77 ± 0.21	0.93 ± 0.21	0.53 ± 0.21	0.73 ± 0.21	0.59 ± 0.21	0.5714	0.8974	0.2625
C18:1	15.4 ± 1.8	15.6 ± 1.8	14.0 ± 1.8	11.3 ± 1.8	12.7 ± 1.8	11.1 ± 1.8	0.575	0.0002	0.2027
C20:1 n-9	0.10 ± 0.14 #	0.46 ± 0.15	0.27 ± 0.14	0.26 ± 0.15	0.13 ± 0.14 *	0.55 ± 0.14	**0.0883**	0.8174	0.2417
C22:1 n-9	0.06 ± 0.05	0.09 ± 0.05	0.06 ± 0.05	0.11 ± 0.05	0.14 ± 0.05	0.02 ± 0.05	0.7994	0.9637	0.138
C24:1 n-9	0.41 ± 0.06	0.38 ± 0.06	0.39 ± 0.06	0.32 ± 0.06	0.40 ± 0.06	0.30 ± 0.06	0.3655	0.596	0.781
MUFA	16.9 ± 1.8	17.4 ± 1.8	15.9 ± 1.8	12.7 ± 1.8	14.4 ± 1.8	12.7 ± 1.8	0.5564	< 0.0001	0.057
C18:2 n-6	18.9 ± 3.0	20.7 ± 3.0	22.4 ± 3.0	19.2 ± 3.0	20.8 ± 3.0	18.5 ± 3.0	0.7452	0.7725	0.305
C18:3 n-6	0.19 ± 0.11	0.16 ± 0.11	0.31 ± 0.11	0.23 ± 0.11	0.51 ± 0.11	0.36 ± 0.11	0.3553	0.0627	0.8472
C20:3 n-6	1.33 ± 0.20	1.50 ± 0.20	1.22 ± 0.20	1.18 ± 0.20	1.67 ± 0.20	1.40 ± 0.20	0.7935	0.1977	0.5041
C20:4 n-6	1.61 ± 0.19	1.58 ± 0.19	1.39 ± 0.19	1.40 ± 0.19	1.64 ± 0.19	1.32 ± 0.19	0.4955	0.5494	0.6125
n-6	22.1 ± 3.0	23.9 ± 3.0	25.3 ± 3.0	22.0 ± 3.0	24.7 ± 3.0	21.6 ± 3.0	0.6954	0.9106	0.2143
C18:3 n-3	2.17 ± 0.31	1.83 ± 0.31	2.57 ± 0.31 *	1.58 ± 0.31	2.26 ± 0.31 *	1.39 ± 0.31	**0.0698**	0.4053	0.1979
C20:5 n-3	**0.81 ± 0.10 ***	**0.51 ± 0.10**	**0.83 ± 0.10 ***	**0.43 ± 0.10**	**0.87 ± 0.10 ***	**0.48 ± 0.10**	**0.0079**	0.8114	0.6777
C22:5 n-3	0.73 ± 0.10	0.77 ± 0.10	0.81 ± 0.10	0.60 ± 0.10	0.78 ± 0.10	0.67 ± 0.10	0.4056	0.8385	0.2796
C22:6 n-3	**0.18 ± 0.07**	**0.02 ± 0.07**	**0.19 ± 0.02**	**0.07 ± 0.02**	**0.19 ± 0.02**	**0.03 ± 0.02**	**0.0753**	0.8726	0.9374
n-3	3.9 ± 0.4	3.1 ± 0.4	4.4 ± 0.4 *	2.7 ± 0.4	4.1 ± 0.4 *	2.6 ± 0.4	**0.025**	0.6813	0.163
PUFA	25.9 ± 3.3	27.1 ± 3.3	29.7 ± 3.3	24.7 ± 3.3	28.8 ± 3.3	24.2 ± 3.3	0.5117	0.9031	0.1918
ratio 16:0 / 16:1	86.6 ± 24.8	65.9 ± 24.8	55.1 ± 24.8	84.1 ± 24.8	93.9 ± 24.8	117.2 ± 24.8	0.6292	0.2939	0.5324
ratio 18:0 / 18:1	2.03 ± 5.56	1.93 ± 5.56	2.24 ± 5.56	13.23 ± 5.56	5.88 ± 5.56	17.51 ± 5.56	0.2506	0.0704	0.2821

ratio n-6 / n-3	5.7 ± 1.9	7.8 ± 1.9	5.8 ± 1.9	7.9 ± 1.9	7.5 ± 1.9 #	13.0 ± 1.9	**0.0301**	0.1611	0.6416

n-3: n-3 polyunsaturated fatty acids; n-6: n-6 polyunsaturated fatty acids; week of suppl°: week of supplementation; SFA = sum of saturated FA; MUFA = sum of monounsaturated FA; PUFA = sum of polyunsaturated FA; n-3= sum of n-3 FA; and n-6 = sum of n-6 FA; C20:5 n-3: eicosapentaenoic acid (EPA); C22:6 n-3: docosahexaenoic acid (DHA); # indicates within a row and a stage a tendency ($p < 0.1$) and * indicates a significant difference between both diets ($p < 0.05$).

Supplementary Table 3. Effect of a PUFA n-3 (n=60) or PUFA n-6 diet (n=61) on lipid abundance in individual oocytes. Mean ± SEM of normalized peak height values

Feature m/z	p-value	PUFA n-6 mean± SEM	Mean n-3± SEM	ratio n3/n6	Feature	p-value	PUFA n-6 mean± SEM	Mean n-3± SEM	ratio n3/n6
m/z 166.16	1.74E-09	129 ± 14	33 ± 2	0.259	m/z 169.21	0.00E+00	233 ± 17	917 ± 65	3.921
m/z 167.20	0.00E+00	243 ± 14	40 ± 2	0.165	m/z 179.20	3.76E-06	243 ± 12	735 ± 98	3.019
m/z 174.15	3.91E-04	580 ± 89	238 ± 12	0.409	m/z 189.17	0.00E+00	68 ± 3	215 ± 10	3.136
m/z 175.17	1.71E-12	6406 ± 747	293 ± 34	0.045	m/z 190.14	0.00E+00	96 ± 5	10340 ± 1064	106.8
m/z 191.08	1.63E-05	613 ± 120	50 ± 3	0.082	m/z 199.22	1.08E-09	50 ± 2	451 ± 59	8.916
m/z 196.12	1.10E-04	771 ± 162	93 ± 7	0.121	m/z 202.16	0.00E+00	38 ± 2	109 ± 6	2.834
m/z 219.21	0.00E+00	8509 ± 721	46 ± 2	0.005	m/z 212.09	5.39E-06	109 ± 19	721 ± 124	6.595
m/z 227.19	2.31E-14	292 ± 25	55 ± 4	0.191	m/z 229.70	0.00E+00	70 ± 4	9330 ± 866	132.0
m/z 243.20	0.00E+00	4710 ± 348	33 ± 1	0.007	m/z 237.24	0.00E+00	28 ± 1	215 ± 15	7.650
m/z 244.20	0.00E+00	1251 ± 112	60 ± 3	0.048	m/z 239.18	0.00E+00	48 ± 2	151 ± 9	3.090
m/z 245.21	0.00E+00	1100 ± 55	73 ± 3	0.066	m/z 247.37	0.00E+00	71 ± 3	5073 ± 366	70.87
m/z 257.22	0.00E+00	724 ± 40	106 ± 8	0.147	m/z 248.38	0.00E+00	53 ± 4	1122 ± 69	20.94
m/z 261.21	0.00E+00	1205 ± 65	79 ± 3	0.066	m/z 251.12	0.00E+00	188 ± 37	1060 ± 56	5.638
m/z 269.22	0.00E+00	898 ± 76	103 ± 6	0.115	m/z 265.20	0.00E+00	101 ± 5	766 ± 53	7.514
m/z 271.24	0.00E+00	6886 ± 435	127 ± 8	0.018	m/z 266.25	0.00E+00	454 ± 26	1191 ± 69	2.622
m/z 281.24	0.00E+00	2225 ± 209	44 ± 1	0.019	m/z 274.38	0.00E+00	175 ± 9	491 ± 29	2.805
m/z 284.28	1.40E-10	1014 ± 124	104 ± 4	0.102	m/z 277.22	0.00E+00	45 ± 1	825 ± 66	18.07
m/z 287.23	0.00E+00	43724 ± 2726	195 ± 14	0.004	m/z 279.22	0.00E+00	196 ± 11	6951 ± 425	35.29
m/z 303.21	0.00E+00	2186 ± 132	595 ± 52	0.272	m/z 295.19	4.39E-13	299 ± 17	2419 ± 254	8.081
m/z 309.20	0.00E+00	809 ± 51	254 ± 12	0.314	m/z 304.41	0.00E+00	1451 ± 158	46978 ± 2640	32.35
m/z 321.24	0.00E+00	3838 ± 260	105 ± 4	0.027	m/z 311.21	0.00E+00	233 ± 11	2089 ± 132	8.936
m/z 324.93	0.00E+00	492 ± 31	84 ± 5	0.172	m/z 312.46	1.20E-08	338 ± 39	1312 ± 150	3.878

m/z 326.47	1.07E-03	2149 ± 589	91 ± 4	0.042	m/z 316.42	0.00E+00	108 ± 5	879 ± 49	8.110
m/z 329.17	0.00E+00	1689 ± 104	482 ± 27	0.285	m/z 317.20	0.00E+00	102 ± 9	243 ± 8	2.370
m/z 343.14	3.66E-08	2369 ± 357	183 ± 6	0.077	m/z 318.22	0.00E+00	104 ± 9	291 ± 12	2.782
m/z 350.21	0.00E+00	529 ± 33	117 ± 4	0.221	m/z 327.47	0.00E+00	849 ± 108	3992 ± 285	4.700
m/z 358.10	0.00E+00	6639 ± 641	222 ± 15	0.033	m/z 332.42	1.04E-02	473 ± 70	2125 ± 611	4.484
m/z 359.12	2.05E-15	6113 ± 604	341 ± 19	0.055	m/z 335.20	3.75E-11	128 ± 8	958 ± 111	7.436
m/z 365.20	0.00E+00	69951 ± 5264	9607 ± 1088	0.137	m/z 336.18	7.05E-13	116 ± 11	244 ± 10	2.099
m/z 369.50	9.50E-04	730 ± 180	93 ± 4	0.128	m/z 337.19	0.00E+00	288 ± 12	1672 ± 110	5.806
m/z 381.18	9.88E-14	5427 ± 584	316 ± 24	0.058	m/z 338.23	3.07E-04	159 ± 7	428 ± 70	2.687
m/z 385.70	1.65E-03	249 ± 43	102 ± 4	0.410	m/z 341.48	9.19E-09	137 ± 21	294 ± 11	2.140
m/z 387.19	7.63E-13	390 ± 28	145 ± 5	0.372	m/z 354.11	4.08E-06	275 ± 16	1885 ± 325	6.841
m/z 395.20	0.00E+00	215 ± 8	101 ± 4	0.469	m/z 362.19	6.42E-14	77 ± 3	467 ± 44	6.069
m/z 397.23	8.41E-12	830 ± 69	275 ± 12	0.331	m/z 368.51	9.71E-08	1644 ± 668	8951 ± 1059	5.444
m/z 398.75	9.50E-04	674 ± 154	130 ± 5	0.192	m/z 371.15	1.07E-15	288 ± 17	1012 ± 73	3.512
m/z 401.22	5.38E-05	348 ± 52	117 ± 4	0.337	m/z 376.41	0.00E+00	296 ± 30	71088 ± 6435	240.1
m/z 405.07	1.04E-06	408 ± 54	106 ± 14	0.261	m/z 378.36	3.04E-15	97 ± 4	809 ± 76	8.329
m/z 419.20	0.00E+00	612 ± 41	59 ± 2	0.097	m/z 379.21	0.00E+00	146 ± 5	591 ± 35	4.028
m/z 423.18	2.05E-15	239 ± 14	95 ± 4	0.398	m/z 380.21	8.68E-07	98 ± 4	399 ± 56	4.035
m/z 438.33	7.13E-03	430 ± 94	156 ± 10	0.364	m/z 391.46	4.95E-15	137 ± 6	3896 ± 409	28.38
m/z 461.16	7.90E-04	2491 ± 598	344 ± 18	0.138	m/z 394.21	3.28E-07	127 ± 6	672 ± 98	5.258
m/z 468.33	3.65E-06	362 ± 47	119 ± 5	0.331	m/z 400.48	0.00E+00	79 ± 8	228 ± 8	2.877
m/z 494.62	6.96E-03	557 ± 155	110 ± 5	0.197	m/z 403.19	0.00E+00	168 ± 12	964 ± 66	5.724
m/z 495.12	4.72E-03	841 ± 189	269 ± 13	0.320	m/z 404.45	2.38E-04	124 ± 17	342 ± 53	2.743
m/z 496.40	0.00E+00	4354 ± 351	613 ± 30	0.140	m/z 413.16	3.48E-08	109 ± 9	429 ± 52	3.935
m/z 498.33	0.00E+00	330 ± 15	123 ± 4	0.374	m/z 414.52	0.00E+00	63 ± 5	476 ± 26	7.529
m/z 517.13	2.05E-15	852 ± 70	176 ± 7	0.206	m/z 447.21	5.53E-07	125 ± 5	457 ± 61	3.646
m/z 522.64	9.13E-06	1985 ± 368	211 ± 15	0.106	m/z 451.20	0.00E+00	96 ± 4	281 ± 14	2.925

m/z 524.44	2.16E-06	511 ± 58	207 ± 7	0.406	m/z 452.53	0.00E+00	80 ± 6	286 ± 17	3.567
m/z 527.20	1.31E-10	21254 ± 2872	297 ± 23	0.014	m/z 481.20	1.45E-03	534 ± 30	1253 ± 213	2.345
m/z 533.17	1.67E-13	8239 ± 861	781 ± 59	0.094	m/z 493.67	1.51E-09	111 ± 5	277 ± 24	2.497
m/z 543.18	1.46E-10	1753 ± 195	325 ± 13	0.185	m/z 505.19	1.29E-11	168 ± 8	443 ± 34	2.631
m/z 577.56	5.98E-13	353 ± 24	137 ± 7	0.388	m/z 508.43	6.25E-13	242 ± 33	4350 ± 497	17.97
m/z 603.56	1.03E-04	364 ± 51	148 ± 6	0.408	m/z 536.17	0.00E+00	181 ± 37	1811 ± 145	9.983
m/z 609.55	3.82E-05	331 ± 45	121 ± 11	0.367	m/z 545.18	4.97E-12	340 ± 23	16378 ± 2049	48.10
m/z 617.11	2.73E-07	336 ± 35	134 ± 5	0.401	m/z 547.19	1.68E-12	133 ± 6	6411 ± 780	47.89
m/z 623.16	6.81E-04	1139 ± 225	304 ± 46	0.267	m/z 559.25	0.00E+00	154 ± 6	455 ± 27	2.949
m/z 640.64	1.27E-05	644 ± 83	244 ± 12	0.378	m/z 561.18	7.84E-12	122 ± 5	528 ± 52	4.307
m/z 642.61	9.98E-05	588 ± 98	175 ± 7	0.298	m/z 562.55	5.19E-05	132 ± 19	266 ± 23	2.007
m/z 650.42	1.10E-11	556 ± 45	172 ± 18	0.310	m/z 579.21	1.39E-05	183 ± 10	419 ± 49	2.285
m/z 666.39	5.09E-12	592 ± 55	143 ± 9	0.242	m/z 594.40	1.89E-10	146 ± 8	396 ± 33	2.705
m/z 668.67	8.49E-05	695 ± 108	229 ± 20	0.330	m/z 639.14	5.18E-03	226 ± 15	504 ± 93	2.228
m/z 677.04	4.93E-12	360 ± 25	144 ± 10	0.401	m/z 654.65	3.70E-03	139 ± 9	284 ± 46	2.041
m/z 688.48	2.25E-13	232 ± 13	108 ± 5	0.465	m/z 659.59	1.49E-02	139 ± 5	395 ± 100	2.825
m/z 689.20	2.38E-07	2367 ± 376	221 ± 16	0.093	m/z 664.59	4.52E-11	176 ± 13	654 ± 63	3.712
m/z 703.57	1.06E-13	7508 ± 663	1452 ± 206	0.193	m/z 679.10	1.02E-07	225 ± 13	710 ± 82	3.144
m/z 706.53	0.00E+00	3961 ± 340	245 ± 23	0.062	m/z 681.17	1.22E-07	186 ± 11	910 ± 125	4.884
m/z 718.55	0.00E+00	480 ± 33	125 ± 5	0.261	m/z 702.52	1.54E-13	124 ± 7	267 ± 14	2.151
m/z 719.56	1.20E-10	370 ± 23	184 ± 10	0.497	m/z 709.15	2.50E-07	114 ± 5	411 ± 52	3.590
m/z 720.55	0.00E+00	2372 ± 188	130 ± 6	0.055	m/z 716.49	0.00E+00	217 ± 24	7875 ± 774	36.15
m/z 725.54	4.55E-11	1547 ± 133	428 ± 66	0.276	m/z 717.57	0.00E+00	258 ± 21	3935 ± 371	15.20
m/z 732.54	1.41E-09	5436 ± 451	2179 ± 161	0.401	m/z 738.58	2.06E-12	119 ± 6	1849 ± 216	15.45
m/z 734.55	0.00E+00	19068 ± 1686	213 ± 9	0.011	m/z 739.56	2.05E-14	161 ± 9	564 ± 44	3.497
m/z 748.56	0.00E+00	1438 ± 130	119 ± 6	0.083	m/z 740.54	7.63E-13	167 ± 8	520 ± 42	3.099

m/z 758.54	0.00E+00	10011 ± 797	1476 ± 110	0.147
m/z 760.57	0.00E+00	28790 ± 2199	165 ± 11	0.005
m/z 780.53	5.43E-09	1542 ± 134	593 ± 57	0.384
m/z 782.54	0.00E+00	5863 ± 432	444 ± 34	0.075
m/z 784.56	0.00E+00	3560 ± 254	787 ± 50	0.221
m/z 786.58	0.00E+00	4961 ± 367	1225 ± 95	0.247
m/z 788.59	0.00E+00	1668 ± 134	277 ± 17	0.166
m/z 804.52	2.40E-10	733 ± 56	295 ± 22	0.402
m/z 808.55	0.00E+00	1587 ± 109	436 ± 37	0.275
m/z 810.58	7.12E-11	1112 ± 77	487 ± 32	0.438
m/z 813.65	6.57E-12	599 ± 44	236 ± 10	0.394
m/z 815.67	5.97E-14	432 ± 33	129 ± 6	0.299
m/z 893.52	4.12E-12	291 ± 23	99 ± 4	0.343
m/z 909.49	2.56E-11	232 ± 15	110 ± 5	0.474
m/z 950.51	2.42E-10	378 ± 34	124 ± 6	0.329
m/z 966.48	4.58E-12	223 ± 17	81 ± 3	0.363

m/z 744.55	0.00E+00	527 ± 34	6213 ± 539	11.78
m/z 746.56	0.00E+00	2165 ± 164	18983 ± 1490	8.764
m/z 766.52	1.11E-06	223 ± 11	554 ± 62	2.483
m/z 768.55	7.94E-06	515 ± 33	1177 ± 134	2.284
m/z 770.54	2.05E-14	407 ± 21	2654 ± 251	6.520
m/z 772.55	0.00E+00	808 ± 55	8746 ± 708	10.81
m/z 774.59	0.00E+00	1050 ± 76	32854 ± 2308	31.28
m/z 794.55	2.93E-09	279 ± 17	1605 ± 201	5.749
m/z 796.54	7.03E-14	433 ± 26	6601 ± 711	15.24
m/z 798.52	0.00E+00	499 ± 38	3419 ± 273	6.846
m/z 800.55	0.00E+00	222 ± 12	4600 ± 343	20.70
m/z 802.51	0.00E+00	123 ± 6	1843 ± 134	14.93
m/z 821.18	3.96E-09	219 ± 19	827 ± 91	3.761
m/z 823.47	6.77E-15	213 ± 19	1859 ± 179	8.695
m/z 824.50	0.00E+00	213 ± 14	1058 ± 82	4.971
m/z 825.52	2.37E-14	121 ± 6	735 ± 68	6.069
m/z 826.52	2.49E-13	115 ± 9	509 ± 45	4.421
m/z 859.15	7.59E-07	139 ± 7	279 ± 25	2.010
m/z 865.18	4.89E-14	92 ± 4	249 ± 17	2.686
m/z 943.57	8.60E-15	80 ± 3	182 ± 10	2.274
m/z 962.50	7.68E-11	92 ± 5	204 ± 14	2.216
m/z 976.52	2.37E-12	122 ± 6	308 ± 22	2.512

Supplementary Table 4. Identification of lipid features detected by MALDI-TOF MS profiling in the oocytes after PUFA n-3 (n = 9 cows) or n-6 (n = 9 cows) diet

Feature m/z	Lipid ID	adduct	m/z theoretical	Delta theoretical/observed	ratio n3/n6 relative abundance
m/z 496.40	LPC (16:0)	[M+H]+	496.3398	-0.0602	0.141
m/z 524.44	LPC (18:0)	[M+H]+	524.3711	-0.0689	0.406
m/z 594.4	LPC (22:4)	[M+Na]+	594.3530	-0.0470	2.706
m/z 703.57	SM (d34:1)	[M+H]+	703.5748	0.0048	0.194
m/z 703.57	CE (20:0)	[M+Na]+	703.6363	0.0663	
m/z 706.53	PC (30:0)	[M+H]+	706.5381	0.0081	0.062
m/z 706.53	PC (O-31:0)	[M+H]+	706.5745	0.0445	
m/z 717.57	SM (d35:1)	[M+H]+	717.5905	0.0205	15.205
m/z 717.57	CE (20:1)	[M+K]+	717.5946	0.0246	
m/z 718.55	PE (34:1)	[M+H]+	718.5381	-0.0119	0.261
m/z 718.55	PC (31:1)	[M+H]+	718.5381	-0.0119	
m/z 718.55	PC (O-32:1) / PC (P-32:0)	[M+H]+	718.5745	0.0245	
m/z 720.55	PC (31:0)	[M+H]+	720.5538	0.0038	0.055
m/z 720.55	PC (O-32:0)	[M+H]+	720.5902	0.0402	
m/z 725.54	SM (d34:1)	[M+Na]+	725.5568	0.0168	0.277
m/z 725.54	SM (d33:2)	[M+K]+	725.4994	-0.0406	
m/z 732.54	PC (32:1)	[M+H]+	732.5538	0.0138	0.401
m/z 732.54	PC (O-33:1) / PC (P-33:0)	[M+H]+	732.5902	0.0502	
m/z 734.55	PC (32:0)	[M+H]+	734.5694	0.0194	0.011
m/z 734.55	PC (31:4)	[M+Na]+	734.4731	-0.0769	
m/z 739.56	SM (d35:1)	[M+Na]+	739.5724	0.0124	3.498
m/z 739.56	SM (d34:2)	[M+K]+	739.5151	-0.0449	
m/z 739.56	CE 22:4	[M+K]+	739.5790	0.0190	
m/z 740.54	PC (31:1)	[M+Na]+	740.5201	-0.0199	3.099
m/z 740.54	PE (34:1)	[M+Na]+	740.5201	-0.0199	
m/z 740.54	PC (O-32:1) / PC (P-32:0)	[M+Na]+	740.5560	0.016	
m/z 744.55	PE (36:2)	[M+H]+	744.5538	0.0038	11.789
m/z 744.55	PC (30:0)	[M+K]+	744.494	-0.056	
m/z 744.55	PC (O-31:0)	[M+K]+	744.5305	-0.0195	
m/z 746.56	PE (36:1)	[M+H]+	746.5694	0.0094	8.764
m/z 746.56	PC (33:1)	[M+H]+	746.5694	0.0094	
m/z 746.56	PC (O-34:1) / PC (P-34:0)	[M+H]+	746.6058	0.0458	
m/z 746.56	PC (O-33:5) / PC (P-33:4)	[M+Na]+	746.5101	-0.0499	
m/z 748.56	PC(33:0)	[M+H]+	748.5851	0.0251	0.083
m/z 748.56	PC (O-35:7) / PC (P-35:6)	[M+H]+	748.5281	-0.0319	

m/z 748.56	PC (O-34:0)	[M+H]+	748.6215	0.0615	
m/z 748.56	PC (O-33:4) / PC (P-33:3)	[M+Na]+	748.5257	-0.0343	
m/z 758.54	PC(34:2)	[M+H]+	758.5694	0.0294	0.147
m/z 758.54	PC (O-35:2) / PC (P-35:1)	[M+H]+	758.6058	0.0658	
m/z 758.54	PC (31:0)	[M+K]+	758.5097	-0.0303	
m/z 758.54	PC (O-32:0)	[M+K]+	758.5460	0.0060	
m/z 760.57	PC(34:1)	[M+H]+	760.5848	0.0148	0.006
m/z 760.57	PC (33:5)	[M+Na]+	760.4888	-0.0812	
m/z 760.57	PC (O-34:5) / PC (P-34:4)	[M+Na]+	760.5252	-0.0448	
m/z 760.57	PE (36 :5)	[M+Na]+	760.488	-0.0812	
m/z 766.52	PE (36:2)	[M+Na]+	766.5357	0.0157	2.483
m/z 766.52	PC (33:2)	[M+Na]+	766.5357	0.0157	
m/z 766.52	PC (35:5)	[M+H]+	766.5381	0.0181	
m/z 766.52	PC (O-36:5) / PC (P-36:4)	[M+H]+	766.5745	0.0545	
m/z 766.52	PC (O-34:2) / PC (P-34:1)	[M+Na]+	766.5721	0.0521	
m/z 766.52	PC (O-33:3) / PC (P-33:2)	[M+K]+	766.5147	-0.0053	
m/z 768.55	PC (33:1)	[M+Na]+	768.5514	0.0014	2.285
m/z 768.55	PC (35:4)	[M+H]+	768.5538	0.0038	
m/z 768.55	PC (O-36:4) / PC (P-36:3)	[M+H]+	768.5902	0.0402	
m/z 768.55	PC (O-34:1) / PC (P-34:0)	[M+Na]+	768.5878	0.0378	
m/z 768.55	PC (32:2)	[M+K]+	768.4940	-0.0560	
m/z 768.55	PC (O-33:2) / PC (P-33:1)	[M+K]+	768.5304	-0.0196	
m/z 768.55	PE (36:1)	[M+Na]+	768.5514	0.0014	
m/z 770.54	PC (33:0)	[M+Na]+	770.5670	0.0270	6.520
m/z 770.54	PC (35:3)	[M+H]+	770.5694	0.0294	
m/z 770.54	PC (O-36:3) / PC (P-36:2)	[M+H]+	770.6058	0.0658	
m/z 770.54	PC (O-35:7) / PC (P-35:6)	[M+Na]+	770.5101	-0.0299	
m/z 770.54	PC (O-34:0)	[M+Na]+	770.6034	0.0634	
m/z 770.54	PC (32:1)	[M+K]+	770.5097	-0.0303	
m/z 770.54	PC (O-33:1) / PC (P-33:0)	[M+K]+	770.5460	0.0060	
m/z 772.55	PC (32:0)	[M+K]+	772.5253	-0.0247	10.814
m/z 772.55	PC (35:2)	[M+H]+	772.5851	0.0351	
m/z 772.55	PC (O-36:2) / PC (P-36:1)	[M+H]+	772.6215	0.0715	
m/z 772.55	PC (O-35:6) / PC (P-35:5)	[M+Na]+	772.5257	-0.0243	
m/z 774.59	PC (34:5)	[M+Na]+	774.5044	-0.0856	31.290

m/z 774.59	PE (38:1)	[M+H]+	774.6007	0.0107	
m/z 774.59	PC (35:1)	[M+H]+	774.6007	0.0107	
m/z 774.59	PC (O-36:1) / PC (P-36:0)	[M+H]+	774.6371	0.0471	
m/z 774.59	PC (O-35:5) / PC (P-35:4)	[M+Na]+	774.5414	-0.0486	
m/z 780.53	PC (34:2)	[M+Na]+	780.5514	0.0214	0.384
m/z 780.53	PC (36:5)	[M+H]+	780.5538	0.0238	
m/z 780.53	PC (O-37:5) / PC (P-37:4)	[M+H]+	780.5908	0.0607	
m/z 780.53	PC (O-35:2) / PC (P-35:1)	[M+Na]+	780.5878	0.0578	
m/z 780.53	PC (33:3)	[M+K]+	780.4940	-0.0360	
m/z 780.53	PC (O-34:3) / PC (P-34:2)	[M+K]+	780.5304	0.0004	
m/z 782.54	PC (34:1)	[M+Na]+	782.5670	0.0270	0.076
m/z 782.54	PC (36:4)	[M+H]+	782.5694	0.0294	
m/z 782.54	PC (O-37:4) / PC (P-37:3)	[M+H]+	782.6064	0.0664	
m/z 782.54	PC (33:2)	[M+K]+	782.5097	-0.0303	
m/z 782.54	PC (O-34:2) / PC (P-34:1)	[M+K]+	782.5460	0.0060	
m/z 782.54	PE (36:2)	[M+K]+	782.5097	-0.0303	
m/z 784.56	PC (36:3)	[M+H]+	784.5851	0.0251	0.221
m/z 784.56	PC (O-37:3) / PC (P-37:2)	[M+H]+	784.6215	0.0615	
m/z 784.56	PC (34:0)	[M+Na]+	784.5827	0.0227	
m/z 784.56	PC (33:1)	[M+K]+	784.5253	-0.0347	
m/z 784.56	PC (O-34:1) / PC (P-34:0)	[M+K]+	784.5617	0.0017	
m/z 784.56	PE (36:1)	[M+K]+	784.5253	-0.0347	
m/z 786.58	PC (36:2)	[M+H]+	786.6007	0.0207	0.247
m/z 786.58	PC (35:6)	[M+Na]+	786.5044	-0.0756	
m/z 786.58	PC (O-36:6) / PC (P-36:5)	[M+Na]+	786.5408	-0.0392	
m/z 786.58	PC (33:0)	[M+K]+	786.5410	-0.0390	
m/z 786.58	PC (O-34:0)	[M+K]+	786.5773	-0.0027	
m/z 786.58	PC(O-35:7) / PC(P-35:6)	[M+K]+	786.5271	-0.0529	
m/z 788.59	PC (36:1)	[M+H]+	788.6164	0.0264	0.166
m/z 788.59	PC (35:5)	[M+Na]+	788.5201	-0.0699	
m/z 788.59	PC (O-36:5) / PC (P-36:4)	[M+Na]+	788.5565	-0.0335	
m/z 794.55	PC (35:2)	[M+Na]+	794.5670	0.0170	5.750
m/z 794.55	PC (37:5)	[M+H]+	794.5694	0.0194	
m/z 794.55	PC (O-38:5) / PC (P-38:4)	[M+H]+	794.6058	0.0558	
m/z 794.55	PC (O-36:2) / PC (P-36:1)	[M+Na]+	794.6034	0.0534	
m/z 794.55	PC (34:3)	[M+K]+	794.5097	-0.0403	

m/z 794.55	PC (O-35:3) / PC (P-35:2)	[M+K]+	794.5460	-0.0040	
m/z 796.54	PC (34:2)	[M+K]+	796.5253	-0.0147	15.244
m/z 796.54	PC (35:1)	[M+Na]+	796.5827	0.0427	
m/z 796.54	PC (37:4)	[M+H]+	796.5851	0.0451	
m/z 796.54	PC (O-38:4) / PC (P-38:3)	[M+H]+	796.6215	0.0815	
m/z 796.54	PC (O-36:1) / PC (P-36:0)	[M+Na]+	796.6191	0.0791	
m/z 796.54	PC (O-35:2) / PC (P-35:1)	[M+K]+	796.5617	0.0217	
m/z 796.54	PE (38:1)	[M+Na]+	796.5827	0.0427	
m/z 798.52	PC (34:1)	[M+K]+	798.5410	0.0210	6.846
m/z 798.52	PC (37:3)	[M+H]+	798.6007	0.0807	
m/z 798.52	PC (36:7)	[M+Na]+	798.5044	-0.0156	
m/z 798.52	PC (O-37:7) / PC (P-37:6)	[M+Na]+	798.5414	0.0214	
m/z 798.52	PC (35:0)	[M+Na]+	798.5983	0.0783	
m/z 800.55	PC (36:6) PC(O-37:6) / PC(P-37:5)	[M+Na]+	800.5201	-0.0299	20.707
m/z 800.55	PC (34:0)	[M+Na]+	800.5561	0.0061	
m/z 800.55	PC (37:2)	[M+K]+	800.5566	0.0066	
m/z 800.55		[M+H]+	800.6158	0.0658	
m/z 802.51	PC (36:5) PC (O-37:5) / PC (P-37:4)	[M+Na]+	802.5357	0.0257	14.936
m/z 802.51	PC (35:6)	[M+Na]+	802.5718	0.0618	
m/z 802.51	PC(O-36:6) / PC(P-36:5)	[M+K]+	802.4780	-0.032	
m/z 802.51		[M+K]+	802.5139	0.0039	
m/z 804.52	PC (36:4)	[M+Na]+	804.5514	0.0314	0.402
m/z 804.52	PC (38:7)	[M+H]+	804.5538	0.0338	
m/z 804.52	PC (O-39:7) / PC (P-39:6)	[M+H]+	804.5908	0.0707	
m/z 804.52	PC (O-37:4) / PC (P-37:3)	[M+Na]+	804.5883	0.0683	
m/z 804.52	PC (35:5)	[M+K]+	804.4940	-0.0260	
m/z 804.52	PC (0-36:5) / PC (P-36:4)	[M+K]+	804.5304	0.0104	
m/z 808.55	PC (36:2)	[M+Na]+	808.5827	0.0327	0.275
m/z 808.55	PC (38:5)	[M+H]+	808.5851	0.0351	
m/z 808.55	PC (O-39:5) / PC (P-39:4)	[M+H]+	808.6221	0.0720	
m/z 808.55	PC (35:3)	[M+K]+	808.5253	-0.0247	
m/z 808.55	PC (O-36:3) / PC (P-36:2)	[M+K]+	808.5617	0.0117	
m/z 810.58	PC (36:1)	[M+Na]+	810.5983	0.0183	0.439
m/z 810.58	PC (38:4)	[M+H]+	810.6007	0.0207	
m/z 810.58	PC (35:2)	[M+K]+	810.5410	-0.0390	
m/z 810.58	PC (O-36:2) / PC (P-36:1)	[M+K]+	810.5773	-0.0027	

m/z 813.65	SM (d42:2)	[M+H]+	813.6844	0.0344	0.395
m/z 813.65	TG (47:1)	[M+Na]+	813.6943	0.0443	
m/z 813.65	TG (46:2)	[M+K]+	813.6369	-0.0131	
m/z 815.67	SM (d42:1)	[M+H]+	815.700	0.030	0.299
m/z 815.67	TG (46:1)	[M+K]+	815.6525	-0.0175	0.299
m/z 824.5	PC (36:2)	[M+K]+	824.5566	0.0566	4.972
m/z 826.52	PC (36:1)	[M+K]+	826.5723	0.0523	4.422
m/z 826.52	PC (38:7)	[M+Na]+	826.5357	0.0157	
m/z 826.52	PC (O-39:7) / PC (P-39:6)	[M+Na]+	826.5727	0.0527	