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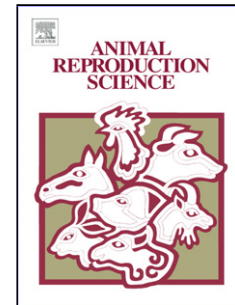
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Effect of a long chain n-3 PUFA-enriched diet on production and reproduction variables in Holstein dairy cows

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1 ABSTRACT

2 The objective of this study was to determine the effect of a rumen-protected fish oil
 3 supplement on the production and reproduction variables in postpartum dairy cows.
 4 Holstein cows ($n = 46$) were given a basal total mixed diet plus one PUFA
 5 supplement: n-3 (n-3; protected fish oil; 1% dry matter intake (DMI); $n = 23$) or
 6 control (n-6; toasted soybeans; 1.8% DMI; $n = 23$), in a switchback design over two
 7 consecutive lactations. Supplements were added to the diet between calving and 2
 8 months after calving to assess the effect on growth and maturation of ovarian follicles
 9 from which ovulation occurred around the day of insemination. Body weight (BW),
 10 milk yield (MY) and composition, dry matter intake (DMI), energy balance (EB),
 11 subcutaneous fat thickness, plasma fatty acid composition, plasma nonesterified fatty
 12 acids (NEFA), glucose and urea concentrations, follicular activity, embryo mortalities
 13 and fertility (conception rate after first AI, AI1) were assessed. BW, MY, DMI,
 14 plasma NEFA, glucose and urea were unaffected by the diet. There was a trend of an
 15 increased number of large follicles (diameter ≥ 10 mm) with the n-3 dietary
 16 supplementation ($P = 0.06$) and a decrease in infertility or early embryo mortality rate
 17 21 days after AI, 13.5% in the n-3 compared with 38.8% in the n-6 group ($P = 0.09$),
 18 with no effect on the conception rate at 35 d or 90 d after AI1. These data suggest that
 19 the effect seen on ovarian variables is not associated with an effect on production and
 20 metabolic variables and is specific to n-3 PUFA supplementation. Further studies are
 21 necessary to determine whether DHA or EPA enhances fertility in lactating dairy
 22 cattle.

23

24 **Keywords:** Dairy cows, Fertility, n-3 PUFA, Nutrition, Production

25

1. Introduction

Reproductive performance in cows is influenced by nutritional and metabolic status, as previously reviewed (Butler, 2000; Roche, 2006; Leroy et al., 2008). Therefore, dietary fat has been used to enhance reproductive status. It is well accepted that dietary fat directly affects dairy cow fertility rather than by modifying energy status of cows (Staples et al., 1998; Mattos et al., 2000). Fatty acids (FA) and polyunsaturated FA (PUFA) were found to be important for various reproductive processes, especially as steroid hormone and prostaglandin precursors via cholesterol and arachidonic acid, respectively (Urlep and Rozman, 2013; Tessaro et al., 2015). Among the PUFA, previous research has focused on the effect of omega-6 (n-6) and especially omega-3 (n-3) FA.

The n-3 PUFA belong to a family of biologically active FA. The n-3 PUFA are essential FA as mammals cannot synthesize n-3 PUFA have to be introduced into the body via the diet. The shortest member of this family, alpha-linolenic acid (ALA, C18:3), has an 18 carbon chain and can be converted by a series of desaturation and elongation reactions into more biologically active compounds (Simopoulos, 2002; Plourde and Cunnane, 2007) of the n-3 family: eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) (Calder, 2012). The ALA can be found in several vegetarian sources, such as grass silage, linseed or dehydrated alfalfa. The conversion rate of ALA into EPA and DHA is low in mammals (Burdge and Calder, 2005). Therefore, to increase concentrations of EPA and DHA in plasma of dairy cows, these two n-3 PUFA have to be directly introduced into the body via the diet. The EPA and DHA are found in fish oil, as well as in microalgae oil (Abughazaleh et al., 2009).

50 The n-3 PUFA have a wide range of physiological roles, via receptors, sensors
 51 or cell membrane fatty acid composition (membrane order, lipid rafts etc...; reviewed
 52 in (Calder, 2012)). Among the physiological roles of n-3 PUFA, there is an effect on
 53 reproductive variables. There is a greater pregnancy rate because of a decrease in
 54 embryo mortality with feeding of a flaxseed diet (Petit and Twagiramungu, 2006).
 55 The feeding of an n-3 diet (enriched in ALA) led to an increase in the size of the pre-
 56 ovulatory follicles (Ambrose et al., 2006) and in number of small follicles (Zachut et
 57 al., 2010). The feeding of diets supplemented with n-3 PUFA (enriched in ALA) also
 58 had a tendency towards increasing conception rates (Ambrose et al., 2006; Dirandeh
 59 et al., 2013). One mechanism that could enhance fertility is that the n-3 diet decreases
 60 the concentration of prostaglandin F2 alpha (PGF2 α) in the uterus, thus potentially
 61 facilitating embryo implantation and reducing embryo mortality (Mattos et al., 2004).
 62 However, some inconsistencies exist, likely due to dose and/or PUFA form
 63 differences, both fish oil (EPA, DHA) and flaxseed (ALA) diets were reported to
 64 decrease corpora lutea (CL) size, and consequently plasma progesterone
 65 concentrations, leading to a more unfavorable uterine environment for sustaining
 66 pregnancies and reduced fertility (Hutchinson et al., 2012).

67 The objective of the present study was, thus, to assess the effect of a moderate
 68 amount of rumen-protected n-3 PUFA on production [body weight (BW), milk yield
 69 (MY), dry matter intake (DMI), energy balance (EB), milk fat content (MFC), milk
 70 protein content (MPC), subcutaneous fat thickness and metabolic variables,
 71 nonesterified fatty acids (NEFA), glucose, urea, as well as on ovarian variables,
 72 growing follicle numbers, conception rate after first AI (AI1)] of 25 Holstein dairy
 73 cows in which assessments occurred over two lactations. Long chain n-3 PUFA
 74 (DHA and EPA) were chosen because these molecules are the most efficient n-3

75 PUFA candidates (Simopoulos, 2002; Deckelbaum and Torrejon, 2012). Rumen-
76 protected fish oil was used to provide DHA and EPA. To ensure a specific effect of
77 long chain n-3 PUFA, an n-6 PUFA diet was chosen as the control. It was
78 hypothesized that the n-3 diet could enhance not only the uterine environment (Mattos
79 et al., 2004) but also oocyte quality, independent of a secondary effect on production
80 or metabolism modifications. Therefore, the diets were fed from calving to 2 months
81 postpartum (2MPP), thus potentially affecting ovarian folliculogenesis and thus the
82 qualitative properties of oocytes released at the ovulation associated with AI1.
83

84 2. Materials and methods

85 2.1. Animals and experimental design

86 All experimental protocols were approved by the local ethics committee
 87 “Comité d’Ethique en Expérimentation Animale Val de Loire (CEEV VdL)”
 88 (protocol registered under ref. 2012-01-1), in accordance with European Directive
 89 2010/63/EU on the protection of animals used for scientific purposes. Holstein cows
 90 ($n = 25$) were managed in loose housing and monitored over 2 consecutive years
 91 (21/25 cows were monitored during the second year) in the same dairy herd in a
 92 switchback design. Each cow received either n-3 or n-6 diets the first year, and the
 93 other diet the following year. For the first year of the experiment, cows were either in
 94 their first ($n = 7$) or second ($n = 18$) lactation. The number of cows used in the
 95 experimental design is presented in Table 1. At the end of the experiment, a total of
 96 23 cows received the n-3 diet and a total of 23 cows received the n-6 diet. Cows
 97 originated from a population previously selected for one female fertility QTL located
 98 on bovine chromosome 3, and carried either a favorable (Fertil+) or unfavorable
 99 (Fertil-) haplotype (Coyral-Castel et al., 2011; Coyral-Castel et al., 2012a). As this
 100 QTL affected reproductive variables during the first lactation, cows with each
 101 haplotype were equally distributed in the two dietary groups (n-3 diet group: 14
 102 Fertil+ and 9 Fertil- cows; n-6 diet group: 15 Fertil+ and 8 Fertil- cows) and the
 103 haplotype effect was taken into account.

104

105 2.2. Experimental diets and dry matter intake

106 The composition of the two experimental diets was balanced for energy and
 107 protein contents as indicated in Table 2; the two diets were total mixed rations
 108 (TMR), with feed values being calculated using the INRA French feeding system

109 (INRA, 2007). The long chain n-3 fatty acid supplement (n-3 diet) was a rumen-
 110 protected encapsulated fish oil OMG750® (Kemin, Nantes, France) and the n-6 fatty
 111 acid supplement (n-6 diet) was toasted soybeans Danex® (Agralys, Blois, France).

112 The supplements were manually mixed into the diet (at 1% DM for n-3 diet
 113 and at 1.8% DM for n-6 diet) and the TMR were distributed twice daily in individual
 114 weighing troughs (Insentec B.V., Marknesse, The Netherlands) as described in
 115 Coyral-Castel et al., 2012b. Cows were fed *ad libitum* and DMI was determined from
 116 the composition of the diets, the feed intake and the dry matter content of each
 117 ingredient included in the diets. The DMI was analyzed daily during the distribution
 118 of the n-3 or n-6 diets, from calving to 2 months postpartum (2MPP).

119 From 2MPP to the non-lactating period, the diet was the same for both groups
 120 and contained (% DM): 68% corn silage, 8% dehydrated alfalfa, 11% Sandifort®
 121 (wheat), 12% Sandilait® (soybean and rapeseed meal), 0.4% vitamins and minerals
 122 5/23 and 0.5% calcium bicarbonate.

124 2.3. Body weight, milk yield and composition, energy balance

125 Body weight (BW) and milk yield (MY) were monitored daily during the
 126 milking period, from calving to 5 months postpartum (5MPP). All cows were milked
 127 twice daily. In the milking parlor, the MY (kg/d) of each cow was automatically
 128 recorded (software Manufeed 500 pro, vc5 version 2.011.14). During the first year of
 129 the experiment, individual milk samples were collected at two consecutive milkings
 130 once a week to determine milk protein content (MPC, g/L) and milk fat content
 131 (MFC, g/L) with Mid-Infrared technics (FOSS, Nanterre, France). After each milking
 132 period, cows were automatically weighed (software RIC version RW1.7). Only the
 133 BW recorded in the morning was used for statistical analysis.

Energy balance (expressed in Mcal/d) was calculated from calving to 2 months postpartum (2MPP) and corresponds to the difference between net energy intake and net energy needs for body maintenance and lactation (cows were inseminated only after 2MPP), according to the INRA method (INRA, 2007). The nutritional values of the different ingredients composing the diets were available from chemical analysis.

140

2.4. Progesterone and metabolic assays, fatty acid composition of plasma and milk

Plasma progesterone was determined using an ELISA assay (Canepa et al., 2008) from 10 μ L of undiluted plasma in samples collected three times a week from 15 days postpartum to the first artificial insemination (AI1) and then daily from the day of AI1 to 35 days after AI1.

For metabolic assays, plasma samples were collected once a week from caudal venipuncture before diet distribution, from 1-month antepartum (1MAP) to 5 months postpartum (5MPP). Plasma samples were stored at -20 °C until the assays were conducted. Plasma NEFA, urea and glucose were determined using enzymatic colorimetry on a multi-parameter analyzer (KONE Instruments Corporation, Espoo, Finland).

Plasma and milk fatty acid compositions were determined using gas chromatography, as previously described (Chajes et al., 2008; Bernard et al., 2009). Plasma fatty acid composition was determined during the 2 years of the experiment for 13 cows in the n-3 diet group and 16 cows in the n-6 diet group, in 1 month antepartum (1MAP), 1 week postpartum (1WPP), 2 month postpartum (2MPP) and 5 month postpartum (5MPP) samples. Milk fatty acid composition was determined in

158 2MPP samples only during the first year of the experiment in samples from six cows
159 in the n-3 diet group and six cows in the n-6 diet group.

160

161 2.5. Subcutaneous adipose tissue thickness

162 Adipose tissue mobilization was assessed through subcutaneous fat thickness
163 measurements in the sacral region using ultrasonographic examination with a linear
164 probe (LA 332 3.5/10.0-MHz transducer; Mylab30vet; Esaote), as previously
165 described (Schroder and Staufenbiel, 2006). Back fat thickness was measured at
166 1MAP, 15WPP, 1MPP, 2MPP and 5MPP during both years of the experiment.

167

168 2.6. Ovarian variables and fertility

169 During the feeding period of n-3 or n-6 diets, the ovarian follicular dynamics
170 of cows ($n = 46$) was monitored three times a week, from 15 ± 2 days postpartum to
171 82 ± 23 days postpartum, with a trans-rectal ultrasonographic examination using a
172 linear probe (LV 513 6.0/8.0-MHz transducer; Mylab30vet; Esaote) LA332 3.5/10.0-
173 MHz, allowing for the detection and measurement of antral follicles, as previously
174 described (Coyral-Castel et al., 2011). Follicles with a diameter of 3 to 5 mm were
175 counted and for all follicles greater than 5 mm, the diameter was recorded. Based on
176 these repeated ultrasonographic examinations, the growth of each follicle was
177 assessed in the two ovaries as previously described (Sirois and Fortune, 1988; Coyral-
178 Castel et al., 2011). Ovarian follicles were classified according to diameter (Lucy et
179 al., 1992): small follicles ($3 \leq SF < 5$ mm), medium follicles ($5 \leq MF < 10$ mm) and
180 large follicles ($LF \geq 10$ mm). To be more accurate in the description of LF, this
181 follicle size category was divided into LFa ($10 \leq LFa < 15$ mm) and LFb ($15 \leq LFb <$
182 25 mm). The mean total number of follicles in each category (SF, MF and LF) was

183 calculated per week postpartum for each cow. Follicular cysts, defined as follicular
184 structures with a diameter larger than 25 mm and from which ovulation did not occur
185 or that did not regress spontaneously, were excluded from the analysis. Numbers of
186 CL were also recorded.

187 Ovulations were determined from two consecutive ultrasonographic
188 examinations (separated by 2 or 3 days): when a pre-ovulatory follicle was detected at
189 one examination, and had disappeared at the subsequent examination. Ovulations
190 were always confirmed by the visualization of CL during examinations that occurred
191 subsequent to the time when ovulation was detected. The first postpartum ovulation
192 was recorded for each cow. For some cows, CL were visualized on the first
193 postpartum examination. Estrous cycle durations were calculated between two
194 consecutive ovulations, and the number of waves of ovarian follicular development
195 was recorded.

196 Cows were artificially inseminated after 60 days postpartum with semen of the
197 same bull, 12 h after visual detection of estrus (observation of standing estrus and/or
198 repetition of secondary signs of estrus). One cow was not inseminated during the first
199 year of the study because she never expressed any signs of estrus.

200 Pregnancy status was determined 21 d after AI1 by plasma progesterone assay
201 (Canepa et al., 2008), and subsequently 35 and 90 d after AI1 using ultrasonographic
202 examination. The percentage of non-fertilization and/or early embryo mortality (NF-
203 EEM) was ascertained by the number of non-pregnant cows at 21 d after AI1 (cows
204 with plasma progesterone < 1 ng/mL) divided by the total number of inseminated
205 cows. The percentage of cows with a prolonged luteal phase or late embryo mortality
206 (PLP-LEM) was ascertained by the number of non-pregnant cows at 35 d divided by
207 the number of cows with plasma progesterone ≥ 1 ng/mL at 21 d after AI. First

208 service conception rate (CRAI1) at 35 and 90 d after AI1 was ascertained by the
 209 number of pregnant cows (ultrasonographic examination) divided by the total number
 210 of inseminated cows. The percentage of cows with late embryo mortality or fetal
 211 mortality (LEM-FM) was ascertained by the number of non-pregnant cows at 90 d
 212 divided by the number of pregnant cows at 35 d. Interval to first insemination (IFI)
 213 was the interval from calving to AI1.

214

215 2.7. Statistical analyses

216 All statistical analyses were performed with SAS® software (SAS Institute
 217 Inc., 2011). Mean milk fatty acid concentrations at 2MPP during the first year of the
 218 experiment were compared between the groups of cows fed the n-3 and n-6 diets
 219 using a Student's t-test (TTEST procedure). All other variables (except NF-EEM,
 220 PLP-LEM, LEM-FM and CRAI1) were analyzed using a linear mixed model
 221 (MIXED procedure) for repeated measurements.

222 For plasma fatty acid composition, the model included the effects of year of
 223 experiment, diet, stage ante/postpartum and all interactions, with stage as a repeated
 224 effect within cow (year) with a CS covariance structure (repeated statement of the
 225 MIXED procedure). Haplotype was not included in the model.

226 For subcutaneous adipose tissue thickness, the model included the effects of
 227 year of experiment, diet, stage ante/postpartum, haplotype and all interactions, with
 228 the subcutaneous adipose tissue thickness value at 1MAP as a covariable, and with
 229 stage as a repeated effect within cow (year) with a CS covariance structure.

230 Concerning the parameters BW, MY, DMI and EB, the model included the
 231 effects of year of experiment, diet, week postpartum (WPP), haplotype and all
 232 interactions, with week postpartum as a repeated effect within cow (year) with a

AR(1) covariance structure and with the value of BW at WPP1 as a covariable for MY and DMI.

For milk composition (MFC and MPC) during the first year of the experiment, the model included the effects of diet, WPP, haplotype and all interactions, with WPP as a repeated effect within cow (year) with a CS covariance structure and with the value of BW at WPP1 as a covariable.

For plasma metabolic variables (NEFA, glucose, urea), the model included the effects of year of experiment, diet, week ante/postpartum (WPP), haplotype and all interactions, with WPP as a repeated effect within cow (year) with a AR(1) covariance structure and with the value of the WPP-4 (week 4 antepartum) as a covariable.

For the follicular growth variables and number of CL, the model included the effects of year of experiment, diet, WPP, haplotype and all interactions, with WPP as a repeated effect within cow (year) with a AR(1) covariance structure.

For the day of first postpartum ovulation, estrous cycle duration, number of follicular waves and IFI, the model included the effects of year of experiment, diet, haplotype and diet x haplotype interaction.

The NF-EEM, PLP-LEM, LEM-FM and CRAI1 were analyzed using a logistic regression (GLIMMIX procedure) and the model included the effects of year of experiment, diet, haplotype and diet x haplotype interaction.

Least square means (lsmeans) estimated by the models were subsequently compared between diets using a Bonferroni adjustment for multiple comparisons. Results are presented in tables and figures as lsmeans \pm SEM, unless otherwise stated. A difference with $P \leq 0.05$ is considered significant and with $0.05 < P \leq 0.10$ is considered a tendency. NS means non-significant for P -values > 0.10 .

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259 3. Results and discussion

260 It was hypothesized that the n-3 diet would enhance oocyte quality
261 independently from a secondary effect on production or metabolism modifications.

262

263 3.1. Efficiency of the diet: Plasma and milk fatty acid composition enrichment in n-3 264 PUFA

265 To assess the efficiency of the n-3 diet, plasma fatty acid composition (Table
266 3) was measured at different stages: before the n-3 diet period (1MAP), during the n-3
267 diet period (1WPP and 2MPP) and after the n-3 diet period (5MPP). As expected,
268 only a few differences were reported between both diets. The EPA (C20:5 n-3) and
269 DHA (C22:6 n-3), both supplied by the n-3 diet, were increased in the n-3 group at
270 both 1WPP ($P = 0.0001$ and $P = 0.01$, respectively) and 2MPP ($P < 0.0001$).
271 Consequently, the n-6 PUFA–n-3 PUFA ratio was less with the n-3 group, at both
272 1WPP and 2MPP, by 29% ($P < 0.0001$) and 41% ($P < 0.0001$), respectively,
273 compared to the n-6 group. Milk fatty acid composition was also assessed at 2MPP
274 during the first year (Table 4). Feeding of both EPA and DHA increased milk
275 composition of these FA more than three-fold ($P < 0.05$).

276 Long chain n-3 EPA and DHA are thought to not be biohydrogenated to a
277 significant extent by rumen microorganisms, except for the C18 n-3 fatty acids (Ashes
278 et al., 1992) but there are inconsistencies in thought in this regard (Castaneda-
279 Gutierrez et al., 2007). Thus, in the present study protected fish oil was chosen so as
280 to maximize the proportion of EPA and DHA in plasma. The rumen-protected form
281 (microencapsulated oil) is sufficient to enable a significant increase in plasma after
282 the first WPP diet, even with the moderate amounts in the diet. The FO diet has been
283 described to increase EPA and DHA in both plasma (Ashes et al., 1992) and milk

(Cant et al., 1997). In addition, FO was reported to decrease the n-6:n-3 ratio in all tissues and milk fat except for subcutaneous and internal adipose tissue (Bilby et al., 2006b), as well as ovarian compartments (Zachut et al., 2010). Therefore, the n-3 diet of the present study might decrease the n-6:n-3 ratio in the reproductive tissues, consequently exerting the physiological effects previously described.

3.2. Production variables: BW, MY, DMI, fat thickness, EB, MFC and MPC

The BW and MY were recorded daily during 22 weeks after calving. The average BW was, throughout the period studied, 593.5 ± 9.2 kg in the n-3 group compared to 586.9 ± 9.1 kg in the n-6 group, with no difference between groups (diet effect $P = 0.61$; Figure 1A). The average MY was, throughout the period studied, 33.0 ± 0.8 kg/d in the n-3 group, compared with 34.4 ± 0.8 kg/d in the n-6 group, with no difference between groups (diet effect $P = 0.25$; Figure 1B).

The MFC and MPC were also assessed once a week during the first year of the experiment for 22 weeks after calving. The average MFC was, throughout the period studied, 46.3 ± 1.8 in the n-3 group compared with 48.5 ± 1.8 g/L in the n-6 group, with no difference between groups (diet effect $P = 0.41$; Figure 1C). The MPC differed between groups ($P = 0.05$) during the first week of lactation (36.5 ± 0.6 compared with 34.3 ± 0.9 g/L milk in the n-3 and n-6 groups, respectively; Figure 1D). Except for the difference reported at WPP1, MPC was not different between the n-3 and n-6 groups (29.7 ± 0.3 and 29.4 ± 0.3 g/L, respectively, diet effect $P = 0.53$).

The DMI and energy balance (EB) were measured during the first 8 weeks postpartum (period of n-3 and n-6 diet distribution). The DMI was not different between groups (diet effect $P = 0.46$), 13.5 ± 0.5 kg DM/day in the n-3 group compared to 14.0 ± 0.5 kg DM / day in the n-6 group (Figure 1E). There was no

309 difference in EB between groups (-11.5 ± 0.8 and -11.6 ± 0.8 kg DM/day in the n-3
310 and n-6 group, respectively, diet effect $P = 0.90$), consistent with the absence of
311 differences for production variables between n-3 and n-6 groups, and all cows were
312 still in negative EB at 2MPP.

313 The production changes (DMI, MY and MFC) that are common with the
314 PUFA diet were not observed in the present study. Unsaturated FA are reported to
315 decrease DMI and increase MY and MFC (reviewed in Rabiee et al., 2012; Leroy et
316 al., 2014)). In a study where supplementation of dairy cow diets with fish oil
317 occurred, it was also reported that there was a tendency to increase MY in the fish oil-
318 treated group (Pirondini et al., 2015). It is common to find a reduction of DMI, MY
319 and changes in milk composition with feeding of the n-3 diet (Lacasse et al., 2002).
320 But with a rumen-protected form of fat, these adverse effects are less frequently
321 encountered (Lacasse et al., 2002). Most meta-analyzed studies compared fat
322 supplementation to the control diet, which was usually enriched with saturated fat to
323 provide an iso-energetic diet (Rabiee et al., 2012), making the conditions of these
324 studies different from conditions in the present study. In the present study, the control
325 diet contained n-6 PUFA, which is the most similar control for an n-3 PUFA diet.
326 Therefore, the absence of differences in production variables in the present study is
327 not surprising.

329 3.3. Fat thickness and plasma metabolic variables

330 To assess mobilization of the body reserve, subcutaneous adipose tissue
331 thickness and plasma NEFA concentrations were measured prepartum and throughout
332 lactation in the present study. Subcutaneous adipose tissue thickness was measured
333 from 1MAP to 5MPP and was 0.46 ± 0.03 cm in the n-3 treatment group compared

with 0.47 ± 0.02 cm in the n-6 treatment group (diet effect $P = 0.71$; Figure 2A). Plasma NEFA, glucose and urea concentrations were recorded from 4 weeks antepartum (WAP-4) to 22WPP. At WPP5, plasma NEFA concentrations were greater in n-3 groups, 1.1 ± 0.1 mM, compared with the n-6 group, 0.8 ± 0.1 mM ($P = 0.039$). There were no other NEFA differences between the groups (diet effect $P = 0.23$). Plasma glucose concentrations were at WAP-4: 3.7 ± 0.1 and 3.9 ± 0.1 mM in the n-3 and n-6 groups, respectively (Figure 2B). Plasma glucose concentrations were different at WPP1 ($P = 0.030$), when there was a decrease to 2.8 ± 0.1 and 2.4 ± 0.1 mM in the n-3 and n-6 groups, respectively. No other plasma glucose concentration differences were detected between the groups (diet effect $P = 0.42$). Plasma urea concentrations increased continuously from WAP-4 to WPP22 (Figure 2C). There was a tendency ($P = 0.098$) for increased plasma urea in the n-3 group at WPP2 compared with the n-6 group (5.5 ± 0.3 and 4.7 ± 0.3 , respectively). There were no other urea differences between the groups (diet effect $P = 0.84$).

The absence of differences in adipose tissue mobilization between both groups is consistent with the absence of a difference in energy balance in the present study. Plasma urea does not exceed the threshold that Butler et al. (1998) described at the time of insemination (19 mg/dL - 6.8 mM - for urea nitrogen in plasma), which can be deleterious for fertility (Butler, 1998). The absence of differences in the glucose concentration between the n-3 and n-6 PUFA groups in the present study is consistent with findings in a previous study comparing diets containing flaxseed and sunflower seed (Ambrose et al., 2006). However, an increased glucose concentration with feeding of a n-3 PUFA diet compared to a n-6 PUFA diet was also reported (Dirandeh et al., 2013).

358 The absence of differences in plasma NEFA concentrations when feeding the
 359 n-3 and n-6 PUFA diets is relevant (Mattos et al., 2004; Mashek et al., 2005). There
 360 are some inconsistencies in results between experiments. As reviewed by Leroy et al.
 361 (2014), fat feeding and fat supplementation increased plasma NEFA as a result of
 362 both DMI reduction and MY and MFC increases, consequently leading to an ovarian
 363 follicular environment that was more unfavorable to cow fertility. The absence of
 364 effects of dietary PUFA on production (DMI, MY, MFC) or metabolism variables
 365 (glucose, NEFA) in the present study is probably related to differences in amount of
 366 PUFA fed, type of dietary fat, and period of feeding of the diet (Leroy et al., 2014)
 367 compared with previous studies. The diet used in the present study provided a
 368 moderate amount of rumen-protected fat (1% DM). Therefore, the lesser amount of
 369 fat and/or the fact that it was rumen-protected could reduce metabolic or production
 370 issues encountered when greater amounts of fat are fed. The control diet in the present
 371 study was an n-6 PUFA diet. The feeding of a saturated or mono-unsaturated diet has
 372 often been used as the control diet in previous studies with results being compared to
 373 those obtained with the n-3 diet. These differences may explain why, in the present
 374 study, there were not any effects on production or metabolic variables.

375

376 3.4. Ovarian variables and fertility at first insemination

377 The first postpartum ovulation occurred before 34.8 ± 5.3 days postpartum in
 378 the n-3 group, and before 32.3 ± 5.3 days postpartum in the n-6 group, with no
 379 difference between them (diet effect $P = 0.74$). Mean estrous cycle duration ($23.3 \pm$
 380 2.7 days in the n-3 group compared with 28.6 ± 2.5 days in the n-6 group, diet effect
 381 $P = 0.15$) and mean number of waves of follicular development per estrous cycle (2.1

382 ± 0.2 in the n-3 group and 2.4 ± 0.2 waves in the n-6 group, respectively; diet effect P
 383 $= 0.26$) were also similar between groups.

384 Follicular growth (monitored by ultrasonographic examination of the ovaries
 385 three times a week) was analyzed from WPP3 to WPP10 (Figure 3). The total
 386 numbers of follicles during this 7 week period were 10.7 ± 0.5 and 10.0 ± 0.5 in the n-
 387 3 and n-6 groups, respectively (Figure 3A; diet effect $P = 0.26$). There was a trend
 388 towards increased follicle numbers at WPP8 in the n-3 group compared with the n-6
 389 group ($P = 0.08$). The numbers of small follicles (SF) were 7.2 ± 0.4 and 6.7 ± 0.4 in
 390 the n-3 and n-6 groups, respectively (Figure 3B; diet effect $P = 0.45$). The numbers of
 391 medium follicles (MF) were 2.4 ± 0.1 and 2.2 ± 0.1 in the n-3 and n-6 groups,
 392 respectively (Figure 3C; diet effect $P = 0.35$). There was a trend for an increased
 393 number of MF at WPP7 in the n-3 group compared with the n-6 group ($P = 0.09$). The
 394 numbers of large follicles (LF) tended to be greater (Figure 3D) in the n-3 group (1.2
 395 ± 0.1) compared with the n-6 group (1.0 ± 0.1 ; diet effect $P = 0.06$) throughout the
 396 period studied and there was a similar trend at both WPP6 and WPP7 ($P = 0.07$). The
 397 LF group was subdivided in two subgroups, LFa ($10 \leq \text{diameter} < 15 \text{ mm}$) and LFb
 398 ($15 \leq \text{diameter} < 25 \text{ mm}$). The numbers of LFa were 0.8 ± 0.0 and 0.7 ± 0.0 in the n-3
 399 and n-6 groups, respectively, and did not differ throughout the period studied (diet
 400 effect $P = 0.14$; Figure 3E). The number of LFa was greater at WPP7 in the n-3 group
 401 compared with the n-6 group ($P = 0.02$). The number of LFb did not differ throughout
 402 the period studied between the n-3 and n-6 groups (0.4 ± 0.0 and 0.3 ± 0.0 ,
 403 respectively, diet effect $P = 0.31$; Figure 3F). However, the number of LFb in the n-3
 404 group at 4WPP tended to increase compared with the n-6 group ($P = 0.09$). The
 405 numbers of CL were 0.9 ± 0.1 and 0.9 ± 0.1 in the n-3 and n-6 groups, respectively
 406 (diet effect $P = 0.74$; Figure 3G).

Pregnancy status was assessed 21, 35 and 90 days after AI1 in the n-3 ($n = 22$) and n-6 groups ($n = 23$). The NF-EEM tended to be less in the n-3 (13.5%) compared with the n-6 group (38.8%, diet effect $P = 0.09$, Table 5). The PLP-LEM was not different between groups: 3/16 cows (15.8%) in the n-3 group and 2/14 cows (14.3%) in the n-6 group (diet effect $P = 0.98$). No difference was observed between groups for CRAI1 (Table 5): 72.9% in the n-3 group and 48.5% in the n-6 group at 35 d (diet effect $P = 0.13$) and 56.1% in the n-3 group and 48.1% in the n-6 group at 90 d after AI1 (diet effect $P = 0.63$). The LEM-FM was not different between groups: 3/16 cows (18.7%) in the n-3 groups and 0/12 cows (0%) in the n-6 group (diet effect $P = 0.98$).

Moreover, there were no differences with the interval to first insemination (IFI) between groups, 80.0 ± 5.1 days in the n-3 group, compared with 84.2 ± 5.0 days in the n-6 group (diet effect $P = 0.56$).

There was a trend for a decrease in NF-EEM in the n-3 group with fish oil supplementation compared to the control group in the present study. This difference is not attributable to a difference in IFI, which is similar in both groups. Rate of NF-EEM of the n-6 group and rates of PLP-LEM of both n-3 and n-6 groups were consistent with results from previous studies, between 20% and 40% and between 8% and 17.5%, respectively (Humblot, 2001; Diskin et al., 2011; Walsh et al., 2011).

These tendencies are consistent with results from previous studies that suggested a potential effect of the n-3 PUFA diet on reproductive variables: an increase in the number of small (Bilby et al., 2006c; Zachut et al., 2010) and medium follicles (Petit et al., 2002) and a trend for decreasing NF-EEM (Ambrose et al., 2006; Petit and Benchaar, 2007; Dirandeh et al., 2013). The differences in numbers of follicles were moderate in the present study, but this finding was expected, as the n-6

431 PUFA diet also affected follicle number in previous research (reviewed in Leroy et
432 al., 2014).

433 Moreover, the experimental design in the present study was meant to focus on
434 oocyte quality, consequently affecting fertilization and/or early embryo development.
435 The diet was provided between calving and 2MPP, thus during folliculogenesis in
436 follicles from which ovulation of oocytes could occur around the time of AI. Potential
437 consequences on oocyte quality can be observed by assessing pregnancy status 21 d
438 and 35 d after AI1 to estimate the rates of NF-EEM and PLP-LEM. Pregnancy
439 failures occurring after 24 d post-AI1 (late embryo or early fetal mortality) are not
440 associated with oocyte quality (Diskin and Morris, 2008; Walsh et al., 2011).

441 Thus, in the present study, benefits of fish oil on NF-EEM could be through
442 oocyte quality enhancement, as hypothesized in the literature (reviewed in Leroy et
443 al., 2014). For example, feeding of an ALA-rich n-3 diet appears to be a satisfactory
444 approach for improving oocyte quality in dairy cows (Moallem et al., 2013). These
445 potential beneficial effects could be amplified as a result of previously described
446 physiologic mechanisms (lesser amounts of PGF2 α , uterine progesterone receptor
447 gene expression increase) that could provide a more favorable uterine environment
448 (Mattos et al., 2004; Bilby et al., 2006a).

449 Fertility at first service can be affected by milk production and metabolic
450 variables which did not occur in the present experiment, where all production and
451 metabolic variables analyzed were similar between groups and not affected by the n-3
452 PUFA diet. However, these data suggest that DHA and EPA provided by the diet
453 might exert direct and specific actions on ovarian function independent of the
454 metabolic and/or production variables.

The difference in NF-EEM between groups was notable but not significant. One explanation is that the treatments imposed on both groups were expected to have beneficial effects on reproduction. This was expected because the n-6 PUFA diet was reported to reduce embryo mortality (Petit and Benchaar, 2007), to increase the number of small (Ponter et al., 2006) and large follicles and estradiol concentrations in the follicular fluid (Zachut et al., 2010) and increase the size of the dominant follicle (Robinson et al., 2002). Feeding a more unsaturated source of FA improved fertilization and embryo development in lactating dairy cows while the metabolic status of the experimental animals was not changed (Cerri et al., 2009). The n-6 PUFA diet is, however, the most desirable control group for the n-3 PUFA diet treatment as both contain polyunsaturated fatty acids, thus a difference in response between groups would indicate a specific effect of n-3 fatty acids. A comparison with a saturated diet will not allow for the discrimination between a beneficial effect of an unsaturated source compared with a PUFA source or a specific effect of n-3 fatty acids. The inclusion of the n-6 PUFA dietary treatment in the present study, as a result of positive effects on reproductive variables, made it more difficult to ascertain the beneficial n-3 dietary effects. Instead of using fish oil to provide EPA and DHA to dairy cows, another strategy could be used by supplying DHA from microalgae, which appears to replace at least in part the DHA from fish oil without leading to any adverse effect on production (Abughazaleh et al., 2009).

475

4. Conclusion

In the present research, effects were studied of a n-3 PUFA diet on production, metabolic and ovarian variables of dairy cows. No differences in production and metabolic variables were detected with the feeding of the n-3 PUFA diet compared

with the control n-6 PUFA diet. An interesting finding in the present study is that the feeding of the n-3 PUFA diet tended to increase the number of large follicles and decrease the non-fertilization and/or early embryo mortality rate. Nevertheless, the conception rate at 35 and 90 d after AI1 was not affected by diet in the present study. These effects do not appear to be related to production and metabolic variables. Effects could, however, be associated with n-3 PUFA-specific dietary effects on the reproductive environment, with the supplementation period used (first 2 months postpartum) suggesting a potential effect on oocyte quality. Further studies are necessary to determine whether DHA or EPA enhances fertility in lactating dairy cattle and to investigate the mechanisms involved.

Declaration of Interest

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

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505

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672 Legends

673

674 Fig. 1. Production variables - Body weight (A) and milk yield (B) were monitored
 675 during 22 weeks postpartum over two lactations (n-3, $n = 23$; n-6, $n = 23$). Milk fat
 676 content (C), milk protein content (D) were monitored during 22 weeks postpartum
 677 over the first lactation only (n-3, $n = 12$; n-6, $n = 12$). Dry matter intake (E) and
 678 energy balance (F) were monitored during 8 weeks postpartum over two lactations (n-
 679 3, $n = 23$; n-6, $n = 23$). Cows received either the n-3 diet (black line) or the n-6 diet
 680 (grey line) from calving to 2 months postpartum. Results are presented as lsmeans +/-
 681 SEM. *indicates a difference ($P < 0.05$); #indicates a tendency ($P < 0.10$)

682

683 Fig. 2. Subcutaneous adipose tissue thickness and metabolic variables - Adipose
 684 tissue mobilization was assessed through subcutaneous fat thickness measurements in
 685 the sacral region using ultrasonographic examination at 1 month antepartum (1MAP),
 686 2 weeks postpartum (2WPP), 1 month postpartum (1MPP), 2 months postpartum
 687 (2MPP) and 5 months postpartum (5MPP) (A). Plasma NEFA (B), glucose (C) and
 688 urea (D) levels were monitored once a week, from 4 weeks antepartum to 22 weeks
 689 postpartum. Cows were monitored over two lactations and received either the n-3 diet
 690 (black line, $n = 23$) or the n-6 diet (grey line, $n = 23$) from calving to 2MPP. Results
 691 are presented as lsmeans +/- SEM. *indicates a difference ($P < 0.05$); #indicates a
 692 tendency ($P < 0.10$).

693

694 Fig. 3. Ovarian follicular dynamics - Total number of follicles (A), the number of
 695 small (B), medium (C) and large (D) follicles as well as the number of CL (G) were
 696 recorded from WPP3 to WPP10. The large follicle category was subdivided into

697 follicles between 10 and 15 mm (E) and follicles between 15 and 25 mm (F). Cows
698 were monitored over two lactations and received either the n-3 diet (black line, $n =$
699 23) or the n-6 diet (grey line, $n = 23$) from calving to 2 months postpartum. Results
700 are presented as lsmeans \pm SEM. *indicates a difference ($P < 0.05$); #indicates a
701 tendency ($P < 0.10$)

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Table 1: Number of cows per diet group and per year

| Diet | Year 1 | Year 2 | Total |
|-------|--------|--------|-------|
| n-3 | 13 | 10 | 23 |
| n-6 | 12 | 11 | 23 |
| Total | 25 | 21 | 46 |

n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids

Table 2: Ingredients composing the experimental diets, and nutritional composition of the diets (mean \pm SEM, $n = 3$ samples)

| | | Diet from calving to 2MPP | |
|--------------------|--|---------------------------|----------------|
| | | n-3 | n-6 |
| Ingredient (% DM): | | | |
| | corn silage | 65.7 | 65.1 |
| | dehydrated alfalfa | 8.3 | 8.3 |
| | Sandifort® (wheat) | 10.3 | 10.2 |
| | Sandilait® (soybean and rapeseed meal) | 13.7 | 13.6 |
| | Vitamins and minerals 5/23 | 0.6 | 0.6 |
| | calcium bicarbonate | 0.4 | 0.4 |
| | encapsulated fish oil | 1 | — |
| | toasted soybeans | — | 1.8 |
| Composition | | | |
| | DM, % | 38.8 \pm 1.6 | 38.4 \pm 1.3 |
| | NE _L , MJ/kg DM | 6.9 \pm 0.1 | 7.0 \pm 0.1 |
| | PDIE, g/kg DM | 91.3 \pm 4.0 | 88.9 \pm 4.4 |
| | PDIN, g/kg DM | 89.0 \pm 6.6 | 86.1 \pm 7.5 |
| | Neutral detergent fiber, % | 32.0 \pm 1.0 | 32.0 \pm 1.4 |
| | Acid detergent fiber, % | 20.8 \pm 0.7 | 20.5 \pm 0.9 |
| | Crude protein, % | 12.8 \pm 0.9 | 12.3 \pm 1.0 |
| | Fat, % | 2.8 \pm 0.2 | 2.7 \pm 0.1 |

n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids; 2MPP, two months postpartum; DM, dry matter; NE_L, net energy for maintenance and lactation; PDIE, intestinal digestible proteins, when Energy is the limiting factor of microbial synthesis; PDIN, intestinal digestible proteins, when degraded N is the limiting factor of microbial synthesis

Table 3: Effect of n-3 ($n = 13$) or n-6 diet ($n = 16$) on plasma fatty acid composition (% of total fatty acid), expressed as lsmeans \pm SEM

| | Stage and diet | | | | | | | |
|-----------------|--------------------|-------------------------------------|-------------------------------------|--------------------------------------|-------------------------------------|-------------------------------------|--------------------|--------------------------------------|
| | 1MAP | | 1WPP | | 2MPP | | 5MPP | |
| | n-3 | n-6 | n-3 | n-6 | n-3 | n-6 | n-3 | n-6 |
| C15:0 | 0.42 \pm 0.01 a | 0.40 \pm 0.01 a | 0.25 \pm 0.01 a | 0.21 \pm 0.01 b | 0.34 \pm 0.01 a | 0.29 \pm 0.01 b | 0.36 \pm 0.02 a | 0.33 \pm 0.01 a |
| C17:0 | 2.73 \pm 0.22 a | 3.35 \pm 0.21 b | 0.92 \pm 0.22 a | 0.83 \pm 0.21 a | 1.77 \pm 0.24 a | 1.62 \pm 0.22 a | 2.62 \pm 0.33 a | 2.32 \pm 0.26 a |
| SFA | 42.55 \pm 0.37 a | 42.75 \pm 0.35 a | 39.75 \pm 0.37 a | 39.63 \pm 0.35 a | 39.25 \pm 0.41 a | 39.58 \pm 0.36 a | 41.08 \pm 0.55 a | 40.15 \pm 0.43 a |
| C14:1 | 0.11 \pm 0.01 a | 0.09 \pm 0.01 b | 0.07 \pm 0.01 a | 0.06 \pm 0.01 a | 0.07 \pm 0.01 a | 0.07 \pm 0.01 a | 0.07 \pm 0.01 a | 0.07 \pm 0.01 a |
| C16:1 | 1.68 \pm 0.09 a | 1.53 \pm 0.09 a | 2.08 \pm 0.09 a | 1.81 \pm 0.09 b | 1.35 \pm 0.10 a | 1.28 \pm 0.09 a | 0.80 \pm 0.12 a | 1.01 \pm 0.10 a |
| C18:1 | 18.74 \pm 0.62 a | 18.38 \pm 0.58 a | 22.97 \pm 0.62 a | 22.86 \pm 0.58 a | 19.05 \pm 0.67 a | 20.96 \pm 0.6 b | 15.43 \pm 0.89 a | 17.84 \pm 0.71 b |
| MUFA | 22.00 \pm 0.65 a | 21.30 \pm 0.60 a | 26.04 \pm 0.65 a | 25.62 \pm 0.60 a | 21.46 \pm 0.70 a | 23.29 \pm 0.63 a | 17.21 \pm 0.92 a | 19.76 \pm 0.73 b |
| C18:2n-6 | 16.58 \pm 0.63 a | 17.35 \pm 0.58 a | 19.33 \pm 0.63 a | 21.55 \pm 0.58 b | 23.93 \pm 0.68 a | 23.68 \pm 0.61 a | 26.86 \pm 0.91 a | 25.00 \pm 0.72 a |
| C20:2n-6 | 0.17 \pm 0.01 a | 0.16 \pm 0.01 a | 0.09 \pm 0.01 a | 0.08 \pm 0.01 a | 0.17 \pm 0.01 a | 0.13 \pm 0.01 b | 0.19 \pm 0.01 a | 0.18 \pm 0.01 a |
| C20:3n-6 | 4.30 \pm 0.19 a | 4.77 \pm 0.18 a | 2.22 \pm 0.19 a | 2.37 \pm 0.18 a | 2.45 \pm 0.21 a | 3.63 \pm 0.18 b | 5.28 \pm 0.27 a | 4.92 \pm 0.22 a |
| C20:3n-9 | 0.22 \pm 0.02 a | 0.26 \pm 0.02 a | 0.17 \pm 0.02 a | 0.18 \pm 0.02 a | 0.14 \pm 0.02 a | 0.21 \pm 0.02 b | 0.20 \pm 0.03 a | 0.24 \pm 0.02 a |
| C20:4n-6 | 5.57 \pm 0.17 a | 5.22 \pm 0.16 a | 4.53 \pm 0.17 a | 4.39 \pm 0.16 a | 3.29 \pm 0.19 a | 3.69 \pm 0.17 a | 3.53 \pm 0.25 a | 3.91 \pm 0.20 a |
| n-6 | 28.29 \pm 0.70 a | 29.03 \pm 0.65 a | 27.4 \pm 0.70 a | 29.43 \pm 0.65 b | 31.12 \pm 0.76 a | 32.38 \pm 0.67 a | 37.21 \pm 1.01 a | 35.67 \pm 0.80 a |
| C18:3n-3 ALA | 2.76 \pm 0.09 a | 2.77 \pm 0.08 a | 1.45 \pm 0.09 a | 1.34 \pm 0.08 a | 1.55 \pm 0.10 a | 1.45 \pm 0.09 a | 1.75 \pm 0.13 a | 1.56 \pm 0.10 a |
| C20:5n-3 EPA | 1.33 \pm 0.15 a | 1.23 \pm 0.14 a | 1.99 \pm 0.15 a | 1.15 \pm 0.14 b | 2.57 \pm 0.16 a | 0.91 \pm 0.14 b | 0.62 \pm 0.22 a | 0.66 \pm 0.17 a |
| C22:6n-3 DHA | 0.50 \pm 0.07 a | 0.46 \pm 0.07 a | 0.79 \pm 0.07 a | 0.52 \pm 0.07 b | 1.42 \pm 0.08 a | 0.32 \pm 0.07 b | 0.26 \pm 0.10 a | 0.16 \pm 0.08 a |
| n-3 | 7.06 \pm 0.30 a | 6.82 \pm 0.28 a | 6.69 \pm 0.30 a | 5.19 \pm 0.28 b | 8.06 \pm 0.33 a | 4.65 \pm 0.29 b | 4.48 \pm 0.43 a | 4.25 \pm 0.35 a |
| PUFA | 35.35 \pm 0.72 a | 35.86 \pm 0.67 a | 34.09 \pm 0.72 a | 34.62 \pm 0.67 a | 39.19 \pm 0.79 a | 37.02 \pm 0.70 b | 41.69 \pm 1.05 a | 39.9 \pm 0.83 a |
| ratio n-6 / n-3 | 5.16 \pm 0.36 a | 5.57 \pm 0.34 a | 5.6 \pm 0.36 a | 7.85 \pm 0.34 b | 5.66 \pm 0.39 a | 9.62 \pm 0.35 b | 11.28 \pm 0.52 a | 11.78 \pm 0.42 a |

n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids; 1MAP, one month antepartum; 1WPP, one week postpartum; 2MPP, two months postpartum; 5MPP, five month postpartum; 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; ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

^{a-b} Means within a row and a stage with different superscripts differ ($P < 0.05$)

Table 4: Effect of n-3 ($n = 6$) or n-6 ($n = 6$) diet on milk fatty acid composition at stage 2MPP during the 1st year of experiment (% of total fatty acid), expressed as means \pm SEM

| Milk FA % | Diet | | Diet effect P-Value |
|---------------------|-------------------------------------|-------------------------------------|------------------------|
| | n-3 | n-6 | |
| C14:0 | 9.54 \pm 0.48 a | 9.41 \pm 0.64 a | 0.88 |
| C15:0 | 1.29 \pm 0.13 a | 0.95 \pm 0.06 b | 0.04 |
| C16:0 | 28.38 \pm 1.47 a | 30.29 \pm 1.23 a | 0.34 |
| SFA | 59.38 \pm 2.21 a | 62.73 \pm 1.77 a | 0.26 |
| C18:1 | 29.03 \pm 1.91 a | 27.61 \pm 1.72 a | 0.59 |
| C20:1 | 0.13 \pm 0.01 a | 0.09 \pm 0.00 b | 0.03 |
| C22:1 | 0.04 \pm 0.01 a | 0.02 \pm 0.00 b | 0.04 |
| MUFA | 33.4 \pm 2.01 a | 31.46 \pm 1.64 a | 0.47 |
| C18:2 | 2.52 \pm 0.11 a | 2.23 \pm 0.11 a | 0.09 |
| C20:2n-6 | 0.04 \pm 0.00 a | 0.03 \pm 0.00 b | 0.02 |
| C20:3n-6 | 0.06 \pm 0.00 a | 0.07 \pm 0.01 b | 0.03 |
| n-6 | 2.74 \pm 0.12 a | 2.46 \pm 0.11 a | 0.13 |
| C18:3n-3 ALA | 0.42 \pm 0.02 a | 0.33 \pm 0.02 b | 0.01 |
| C20:5n-3 EPA | 0.10 \pm 0.02 a | 0.03 \pm 0.00 b | 0.02 |
| C22:6n-3 DHA | 0.07 \pm 0.02 a | 0.02 \pm 0.00 b | 0.03 |
| n-3 | 0.74 \pm 0.08 a | 0.45 \pm 0.02 b | 0.01 |
| PUFA | 3.48 \pm 0.17 a | 2.91 \pm 0.14 b | 0.03 |
| CLA | 0.81 \pm 0.09 a | 0.51 \pm 0.02 b | 0.02 |

n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids; 2MPP, two months postpartum ;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; and CLA = conjugated linoleic acid; ALA, alpha linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

^{a-b} Means within a row with different superscripts differ ($P < 0.05$)

Table 5: Effect of n-3 ($n = 22$) or n-6 ($n = 23$) diet on NF-EEM and CRAI1 (expressed as estimated %)

| | Diet | | Diet effect | |
|----------------------|--------|--------|-------------------------------|---------|
| | n-3 | n-6 | OR (95 % confidence interval) | P-value |
| NF-EEM | 13.50% | 38.80% | 0.245 (0.049- 1.229) | 0.086 |
| CRAI1 35 d after AI1 | 72.90% | 48.50% | 2.861 (0.726- 11.279) | 0.129 |
| CRAI1 90 d after AI1 | 56.10% | 48.10% | 1.377 (0.365- 5.198) | 0.629 |

n-3, n-3 polyunsaturated fatty acids; n-6, n-6 polyunsaturated fatty acids; OR, odds ratio; NF-EEM, non fertilization - early embryo mortality (plasma progesterone 21 d after AI1); CRAI1, conception rate after first AI (ultrasonography)

