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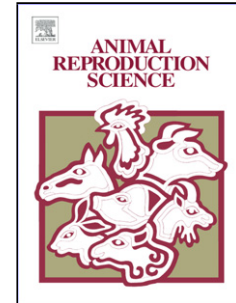
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**Effect of a long chain n-3 PUFA-enriched diet on production and reproduction variables
in Holstein dairy cows**

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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

26 1. Introduction

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

37 The n-3 PUFA belong to a family of biologically active FA. The n-3 PUFA
38 are essential FA as mammals cannot synthesize n-3 PUFA have to be introduced into
39 the body via the diet. The shortest member of this family, alpha-linolenic acid (ALA,
40 C18:3), has an 18 carbon chain and can be converted by a series of desaturation and
41 elongation reactions into more biologically active compounds (Simopoulos, 2002;
42 Plourde and Cunnane, 2007) of the n-3 family: eicosapentaenoic acid (EPA, C20:5)
43 and docosahexaenoic acid (DHA, C22:6) (Calder, 2012). The ALA can be found in
44 several vegetarian sources, such as grass silage, linseed or dehydrated alfalfa. The
45 conversion rate of ALA into EPA and DHA is low in mammals (Burdge and Calder,
46 2005). Therefore, to increase concentrations of EPA and DHA in plasma of dairy
47 cows, these two n-3 PUFA have to be directly introduced into the body via the diet.
48 The EPA and DHA are found in fish oil, as well as in microalgae oil (Abughazaleh et
49 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 (CEEA 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.

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

140

141 *2.4. Progesterone and metabolic assays, fatty acid composition of plasma and milk*

142 Plasma progesterone was determined using an ELISA assay (Canepa et al.,
143 2008) from 10 μ L of undiluted plasma in samples collected three times a week from
144 15 days postpartum to the first artificial insemination (AI1) and then daily from the
145 day of AI1 to 35 days after AI1.

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

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

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

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

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

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

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

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

253 Least square means (lsmeans) estimated by the models were subsequently
254 compared between diets using a Bonferroni adjustment for multiple comparisons.
255 Results are presented in tables and figures as lsmeans \pm SEM, unless otherwise stated.
256 A difference with $P \leq 0.05$ is considered significant and with $0.05 < P \leq 0.10$ is
257 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

284 (Cant et al., 1997). In addition, FO was reported to decrease the n-6:n-3 ratio in all
285 tissues and milk fat except for subcutaneous and internal adipose tissue (Bilby et al.,
286 2006b), as well as ovarian compartments (Zachut et al., 2010). Therefore, the n-3 diet
287 of the present study might decrease the n-6:n-3 ratio in the reproductive tissues,
288 consequently exerting the physiological effects previously described.

289

290 3.2. Production variables: BW, MY, DMI, fat thickness, EB, MFC and MPC

291 The BW and MY were recorded daily during 22 weeks after calving. The
292 average BW was, throughout the period studied, 593.5 ± 9.2 kg in the n-3 group
293 compared to 586.9 ± 9.1 kg in the n-6 group, with no difference between groups (diet
294 effect $P = 0.61$; Figure 1A). The average MY was, throughout the period studied, 33.0
295 ± 0.8 kg/d in the n-3 group, compared with 34.4 ± 0.8 kg/d in the n-6 group, with no
296 difference between groups (diet effect $P = 0.25$; Figure 1B).

297 The MFC and MPC were also assessed once a week during the first year of the
298 experiment for 22 weeks after calving. The average MFC was, throughout the period
299 studied, 46.3 ± 1.8 in the n-3 group compared with 48.5 ± 1.8 g/L in the n-6 group,
300 with no difference between groups (diet effect $P = 0.41$; Figure 1C). The MPC
301 differed between groups ($P = 0.05$) during the first week of lactation (36.5 ± 0.6
302 compared with 34.3 ± 0.9 g/L milk in the n-3 and n-6 groups, respectively; Figure
303 1D). Except for the difference reported at WPP1, MPC was not different between the
304 n-3 and n-6 groups (29.7 ± 0.3 and 29.4 ± 0.3 g/L, respectively, diet effect $P = 0.53$).

305 The DMI and energy balance (EB) were measured during the first 8 weeks
306 postpartum (period of n-3 and n-6 diet distribution). The DMI was not different
307 between groups (diet effect $P = 0.46$), 13.5 ± 0.5 kg DM/day in the n-3 group
308 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

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

348 The absence of differences in adipose tissue mobilization between both groups
349 is consistent with the absence of a difference in energy balance in the present study.
350 Plasma urea does not exceed the threshold that Butler et al. (1998) described at the
351 time of insemination (19 mg/dL - 6.8 mM - for urea nitrogen in plasma), which can be
352 deleterious for fertility (Butler, 1998). The absence of differences in the glucose
353 concentration between the n-3 and n-6 PUFA groups in the present study is consistent
354 with findings in a previous study comparing diets containing flaxseed and sunflower
355 seed (Ambrose et al., 2006). However, an increased glucose concentration with
356 feeding of a n-3 PUFA diet compared to a n-6 PUFA diet was also reported (Dirandeh
357 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 ($10 \leq \text{diameter} < 15$ mm) and LFb
398 ($15 \leq \text{diameter} < 25$ 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).

407 Pregnancy status was assessed 21, 35 and 90 days after AI1 in the n-3 ($n = 22$)
408 and n-6 groups ($n = 23$). The NF-EEM tended to be less in the n-3 (13.5%) compared
409 with the n-6 group (38.8%, diet effect $P = 0.09$, Table 5). The PLP-LEM was not
410 different between groups: 3/16 cows (15.8%) in the n-3 group and 2/14 cows (14.3%)
411 in the n-6 group (diet effect $P = 0.98$). No difference was observed between groups
412 for CRAI1 (Table 5): 72.9% in the n-3 group and 48.5% in the n-6 group at 35 d (diet
413 effect $P = 0.13$) and 56.1% in the n-3 group and 48.1% in the n-6 group at 90 d after
414 AI1 (diet effect $P = 0.63$). The LEM-FM was not different between groups: 3/16 cows
415 (18.7%) in the n-3 groups and 0/12 cows (0%) in the n-6 group (diet effect $P = 0.98$).

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

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

425 These tendencies are consistent with results from previous studies that
426 suggested a potential effect of the n-3 PUFA diet on reproductive variables: an
427 increase in the number of small (Bilby et al., 2006c; Zachut et al., 2010) and medium
428 follicles (Petit et al., 2002) and a trend for decreasing NF-EEM (Ambrose et al., 2006;
429 Petit and Benchaar, 2007; Dirandeh et al., 2013). The differences in numbers of
430 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 PGF 2α , 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.

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

475

476 **4. Conclusion**

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

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

490

491 **Declaration of Interest**

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

494

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505

506

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507 **References**

- 508 Abughazaleh, A.A., Potu, R.B., Ibrahim, S., 2009. Short communication: The effect
509 of substituting fish oil in dairy cow diets with docosahexaenoic acid-micro
510 algae on milk composition and fatty acids profile. *J. Dairy Sci.* 92, 6156-6159.
- 511 Ambrose, D.J., Kastelic, J.P., Corbett, R., Pitney, P.A., Petit, H.V., Small, J.A.,
512 Zalkovic, P., 2006. Lower pregnancy losses in lactating dairy cows fed a diet
513 enriched in alpha-linolenic acid. *J. Dairy Sci.* 89, 3066-3074.
- 514 Ashes, J.R., Siebert, B.D., Gulati, S.K., Cuthbertson, A.Z., Scott, T.W., 1992.
515 Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of
516 ruminants. *Lipids* 27, 629-631.
- 517 Bernard, L., Shingfield, K.J., Rouel, J., Ferlay, A., Chilliard, Y., 2009. Effect of plant
518 oils in the diet on performance and milk fatty acid composition in goats fed
519 diets based on grass hay or maize silage. *Br. J. Nutr.* 101, 213-224.
- 520 Bilby, T.R., Guzeloglu, A., MacLaren, L.A., Staples, C.R., Thatcher, W.W., 2006a.
521 Pregnancy, bovine somatotropin, and dietary n-3 fatty acids in lactating dairy
522 cows: II. Endometrial gene expression related to maintenance of pregnancy. *J.*
523 *Dairy Sci.* 89, 3375-3385.
- 524 Bilby, T.R., Jenkins, T., Staples, C.R., Thatcher, W.W., 2006b. Pregnancy, bovine
525 somatotropin, and dietary n-3 fatty acids in lactating dairy cows: III. Fatty acid
526 distribution. *J. Dairy Sci.* 89, 3386-3399.
- 527 Bilby, T.R., Sozzi, A., Lopez, M.M., Silvestre, F.T., Ealy, A.D., Staples, C.R.,
528 Thatcher, W.W., 2006c. Pregnancy, bovine somatotropin, and dietary n-3 fatty
529 acids in lactating dairy cows: I. Ovarian, conceptus, and growth hormone-
530 insulin-like growth factor system responses. *J. Dairy Sci.* 89, 3360-3374.
- 531 Burdge, G.C., Calder, P.C., 2005. Conversion of alpha-linolenic acid to longer-chain
532 polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.* 45, 581-597.
- 533 Butler, W.R., 1998. Review: effect of protein nutrition on ovarian and uterine
534 physiology in dairy cattle. *J. Dairy Sci.* 81, 2533-2539.
- 535 Butler, W.R., 2000. Nutritional interactions with reproductive performance in dairy
536 cattle. *Anim. Reprod. Sci.* 60-61, 449-457.
- 537 Calder, P.C., 2012. Mechanisms of action of (n-3) fatty acids. *J. Nutr.* 142, 592S-
538 599S.
- 539 Canepa, S., Laine, A.B., A., Fagu, C., Flon, C., Monniaux, D., 2008. Validation d'une
540 methode immunoenzymatique pour le dosage de la progesterone dans le
541 plasma des ovins et des bovins. *Les Cahiers Techniques de L'INRA* 64, 19-30.
- 542 Cant, J.P., Fredeen, A.H., MacIntyre, T., Gunn, J., Crowe, N., 1997. Effect of fish oil
543 and monensin on milk composition in dairy cows. *Can. J. Anim. Sci.* 77, 125-
544 131.
- 545 Castaneda-Gutierrez, E., de Veth, M.J., Lock, A.L., Dwyer, D.A., Murphy, K.D.,
546 Bauman, D.E., 2007. Effect of supplementation with calcium salts of fish oil
547 on n-3 fatty acids in milk fat. *J. Dairy Sci.* 90, 4149-4156.
- 548 Cerri, R.L., Juchem, S.O., Chebel, R.C., Rutigliano, H.M., Bruno, R.G., Galvao,
549 K.N., Thatcher, W.W., Santos, J.E., 2009. Effect of fat source differing in fatty
550 acid profile on metabolic parameters, fertilization, and embryo quality in high-
551 producing dairy cows. *J. Dairy Sci.* 92, 1520-1531.
- 552 Chajes, V., Thiebaut, A.C., Rotival, M., Gauthier, E., Maillard, V., Boutron-Ruault,
553 M.C., Joulin, V., Lenoir, G.M., Clavel-Chapelon, F., 2008. Association
554 between serum trans-monounsaturated fatty acids and breast cancer risk in the
555 E3N-EPIC Study. *Am. J. Epidemiol.* 167, 1312-1320.

- 556 Coyral-Castel, S., Brisard, D., Touze, J.L., Dupont, M., Rame, C., Uzbekova, S.,
 557 Dupont, J., 2012a. Analysis of in vivo oocyte maturation, in vitro embryo
 558 development and gene expression in cumulus cells of dairy cows and heifers
 559 selected for one fertility quantitative trait loci (QTL) located on BTA3.
 560 *Theriogenology* 77, 1822-1833.e1821.
- 561 Coyral-Castel, S., Faverdin, P., Ramé, C., Fréret, S., Guillaume, D., Fritz, S., Dupont,
 562 J., 2012b. Significant differences in fertility between dairy cows selected for
 563 one QTL located on bovine chromosome 3 are not attributable to energy
 564 balance, although eating behaviour is affected. *Animal* 7, 610-617.
- 565 Coyral-Castel, S., Ramé, C., Monniaux, D., Fréret, S., Fabre-Nys, C., Fritz, S.,
 566 Monget, P., Dupont, F., Dupont, J., 2011. Ovarian parameters and fertility of
 567 dairy cows selected for one QTL located on BTA3. *Theriogenology* 75, 1239-
 568 1250.
- 569 Deckelbaum, R.J., Torrejon, C., 2012. The omega-3 fatty acid nutritional landscape:
 570 health benefits and sources. *J. Nutr.* 142, 587S-591S.
- 571 Dirandeh, E., Towhidi, A., Zeinoaldini, S., Ganjkanlou, M., Ansari Pirsaraei, Z.,
 572 Fouladi-Nashta, A., 2013. Effects of different polyunsaturated fatty acid
 573 supplementations during the postpartum periods of early lactating dairy cows
 574 on milk yield, metabolic responses, and reproductive performances. *J. Anim.*
 575 *Sci.* 91, 713-721.
- 576 Diskin, M.G., Morris, D.G., 2008. Embryonic and Early Foetal Losses in Cattle and
 577 Other Ruminants. *Reprod. Domest. Anim.* 43, 260-267.
- 578 Diskin, M.G., Parr, M.H., Morris, D.G., 2011. Embryo death in cattle: an update.
 579 *Reprod. Fertil. Dev.* 24, 244-251.
- 580 Humblot, P., 2001. Use of pregnancy specific proteins and progesterone assays to
 581 monitor pregnancy and determine the timing, frequencies and sources of
 582 embryonic mortality in ruminants. *Theriogenology* 56, 1417-1433.
- 583 Hutchinson, I.A., Hennessy, A.A., Waters, S.M., Dewhurst, R.J., Evans, A.C.O.,
 584 Lonergan, P., Butler, S.T., 2012. Effect of supplementation with different fat
 585 sources on the mechanisms involved in reproductive performance in lactating
 586 dairy cattle. *Theriogenology* 78, 12-27.
- 587 INRA, 2007. Alimentation des bovins, ovins et caprins : Besoins des animaux -
 588 Valeurs des aliments, Paris, France : Quae editions.
- 589 Lacasse, P., Kennelly, J.J., Delbecchi, L., Ahnadi, C.E., 2002. Addition of protected
 590 and unprotected fish oil to diets for dairy cows. I. Effects on the yield,
 591 composition and taste of milk. *J. Dairy Res.* 69, 511-520.
- 592 Leroy, J., Sturmeijer, R.G., Van Hoeck, V., De Bie, J., McKeegan, P.J., Bols, P.E.J.,
 593 2014. Dietary fat supplementation and the consequences for oocyte and
 594 embryo quality: hype or significant benefit for dairy cow reproduction?
 595 *Reprod. Domest. Anim.* 49, 353-361.
- 596 Leroy, J.L., Vanholder, T., Van Kneegsel, A.T., Garcia-Ispuerto, I., Bols, P.E., 2008.
 597 Nutrient prioritization in dairy cows early postpartum: mismatch between
 598 metabolism and fertility? *Reprod. Domest. Anim.* 43 Suppl 2, 96-103.
- 599 Lucy, M.C., Savio, J.D., Badinga, L., De La Sota, R.L., Thatcher, W.W., 1992.
 600 Factors that affect ovarian follicular dynamics in cattle. *J. Anim. Sci.* 70,
 601 3615-3626.
- 602 Mashek, D.G., Bertics, S.J., Grummer, R.R., 2005. Effects of intravenous
 603 triacylglycerol emulsions on hepatic metabolism and blood metabolites in
 604 fasted dairy cows. *J. Dairy Sci.* 88, 100-109.

- 605 Mattos, R., Staples, C.R., Arteché, A., Wiltbank, M.C., Diaz, F.J., Jenkins, T.C.,
 606 Thatcher, W.W., 2004. The effects of feeding fish oil on uterine secretion of
 607 PGF₂α, milk composition, and metabolic status of periparturient Holstein
 608 cows. *J. Dairy Sci.* 87, 921-932.
- 609 Mattos, R., Staples, C.R., Thatcher, W.W., 2000. Effects of dietary fatty acids on
 610 reproduction in ruminants. *Rev. Reprod.* 5, 38-45.
- 611 Moallem, U., Shafran, A., Zachut, M., Dekel, I., Portnick, Y., Arieli, A., 2013.
 612 Dietary alpha-linolenic acid from flaxseed oil improved folliculogenesis and
 613 IVF performance in dairy cows, similar to eicosapentaenoic and
 614 docosahexaenoic acids from fish oil. *Reproduction* 146, 603-614.
- 615 Petit, H.V., Benchaar, C., 2007. Importance of dietary fat on reproduction of dairy
 616 cows. *Renc. Rech. Ruminants* 14, 329-332.
- 617 Petit, H.V., Dewhurst, R.J., Scollan, N.D., Proulx, J.G., Khalid, M., Haresign, W.,
 618 Twagiramungu, H., Mann, G.E., 2002. Milk production and composition,
 619 ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats.
 620 *J. Dairy Sci.* 85, 889-899.
- 621 Petit, H.V., Twagiramungu, H., 2006. Conception rate and reproductive function of
 622 dairy cows fed different fat sources. *Theriogenology* 66, 1316-1324.
- 623 Pirondini, M., Colombini, S., Mele, M., Malagutti, L., Rapetti, L., Galassi, G.,
 624 Crovetto, G.M., 2015. Effect of dietary starch concentration and fish oil
 625 supplementation on milk yield and composition, diet digestibility, and
 626 methane emissions in lactating dairy cows. *J. Dairy Sci.* 98, 357-372.
- 627 Plourde, M., Cunnane, S.C., 2007. Extremely limited synthesis of long chain
 628 polyunsaturates in adults: implications for their dietary essentiality and use as
 629 supplements. *Appl. Physiol. Nutr. Metab.* 32, 619-634.
- 630 Ponter, A.A., Arnault, J., Guelou, K., Ponchon, S., Gonzales, C., Grimard, B.,
 631 Humblot, P., 2006. Effect of the type of dietary fatty acid (α -linolenic acid or
 632 linoleic acid) on the number and the quality of oocytes collected by Ovum
 633 Pick-Up and on the production of embryos by dairy heifers. *Renc. Rech.*
 634 *Ruminants* 13, 289.
- 635 Rabiee, A.R., Breinhild, K., Scott, W., Golder, H.M., Block, E., Lean, I.J., 2012.
 636 Effect of fat additions to diets of dairy cattle on milk production and
 637 components: a meta-analysis and meta-regression. *J. Dairy Sci.* 95, 3225-
 638 3247.
- 639 Robinson, R.S., Pushpakumara, P.G., Cheng, Z., Peters, A.R., Abayasekara, D.R.,
 640 Wathes, D.C., 2002. Effects of dietary polyunsaturated fatty acids on ovarian
 641 and uterine function in lactating dairy cows. *Reproduction* 124, 119-131.
- 642 Roche, J.F., 2006. The effect of nutritional management of the dairy cow on
 643 reproductive efficiency. *Anim. Reprod. Sci.* 96, 282-296.
- 644 SAS institute Inc., 2011. SAS/STAT 9.2 user's Guide, Second edition. Cary, NC: SAS
 645 institute Inc.
- 646 Schroder, U.J., Staufenbiel, R., 2006. Invited review: Methods to determine body fat
 647 reserves in the dairy cow with special regard to ultrasonographic measurement
 648 of backfat thickness. *J. Dairy Sci.* 89, 1-14.
- 649 Simopoulos, A.P., 2002. Omega-3 fatty acids in inflammation and autoimmune
 650 diseases. *J. Am. Coll. Nutr.* 21, 495-505.
- 651 Sirois, J., Fortune, J.E., 1988. Ovarian follicular dynamics during the estrous cycle in
 652 heifers monitored by real-time ultrasonography. *Biol. Reprod.* 39, 308-317.

- 653 Staples, C.R., Burke, J.M., Thatcher, W.W., 1998. Influence of supplemental fats on
654 reproductive tissues and performance of lactating cows. *J. Dairy Sci.* 81, 856-
655 871.
- 656 Tessaro, F.H., Ayala, T.S., Martins, J.O., 2015. Lipid Mediators Are Critical in
657 Resolving Inflammation: A Review of the Emerging Roles of Eicosanoids in
658 Diabetes Mellitus. *Biomed Res Int* 2015, 568408.
- 659 Urlep, Z., Rozman, D., 2013. The Interplay between Circadian System, Cholesterol
660 Synthesis, and Steroidogenesis Affects Various Aspects of Female
661 Reproduction. *Front Endocrinol (Lausanne)* 4, 111.
- 662 Walsh, S.W., Williams, E.J., Evans, A.C., 2011. A review of the causes of poor
663 fertility in high milk producing dairy cows. *Anim. Reprod. Sci.* 123, 127-138.
- 664 Zachut, M., Dekel, I., Lehrer, H., Arieli, A., Arav, A., Livshitz, L., Yakoby, S.,
665 Moallem, U., 2010. Effects of dietary fats differing in n-6:n-3 ratio fed to
666 high-yielding dairy cows on fatty acid composition of ovarian compartments,
667 follicular status, and oocyte quality. *J. Dairy Sci.* 93, 529-545.
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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 +/- 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 $\text{lsmeans} \pm \text{SEM}$

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

