Effects of the precalving administration of omega-3 fatty acids alone or in combination with acetylsalicylic acid in periparturient dairy cows

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ABSTRACT: This study investigated the effects of the administration of long chain omega-3 fatty acids (ω-3 FA) and acetylsalicylic acid (ASA) on inflammation, performance, and fertility in periparturient dairy cows. Five weeks before calving, 26 multiparous dairy cows were randomly assigned to 1 of 3 treatments: ω-3 FA (n = 9; OME), ω-3 FA and ASA (n = 9; OMAS), or palm oil (n = 8; CTR). During the last 3 wk of pregnancy, OME and OMAS groups received daily 12.0 g of fish-derived ω-3 FA, whereas CTR cows received only SFA. In addition, OMAS cows received daily 6.0 mg ASA/kg BW starting at 7 d before calving. Only a few cows had health problems after calving, but those in OMAS were most affected (n = 3 vs. 1 in CTR). Inflammatory status around calving did not improve in OME cows, as confirmed by the patterns of concentration of acute-phase proteins (APP), which were similar to CTR. Compared with CTR and OME, the increase of the positive APP and the decrease of the negative APP (e.g., albumin; P < 0.01) observed in OMAS cows suggested a severe inflammatory status after calving. Compared with OMAS, postcalving energy metabolism was better in OME cows as shown by a lower degree of lipomobilization (smaller BCS drop, greater glucose) and milder ketogenesis (less β-hydroxybutyrate; P < 0.01). Cows in CTR had optimal fertility indices, whereas OMAS was the worst group. The severe inflammation and the more negative energy balance likely contributed to the poor fertility parameters in those cows. It is known that ASA exerts an inhibitory effect on cyclooxygenases, causing a possible decrease in the synthesis of PGF2α. A decreased concentration of PGF2α is connected with alterations in the physiologic processes related to labor and to uterine motility. Cows in OMAS had a longer pregnancy (P < 0.10 vs. OME) and a greater frequency of retained placenta, which may be attributed to decreased synthesis of PGF2α. The administration of ω-3 FA alone did not delay calving or the expulsion of fetal membranes. In conclusion, long-chain ω-3 FA improved the physiological status of cows, partly through better energy balance. The administration of ASA before calving (even at a low dose) in combination with ω-3 FA did not exert any synergistic positive effect on inflammation and performance.

Key words: acetylsalicylic acid, dairy cow, omega-3 fatty acids, transition period

INTRODUCTION

The transition period is defined as the 3 wk before through 3 wk after calving (Grummer, 1995) and it is considered the most critical phase of the life of the dairy cow (Goff and Horst, 1997; Drackley, 1999). The incidence of health problems occurring during this period may increase the risk of developing other diseases, suboptimal milk yield and poor fertility (Bertoni et al., 2008). All cows during the transition period (with or without clinical symptoms) experience some degree of inflammation, and the response of the animal often leads to negative consequences due to the effects of the mediators involved, mainly cytokines and eicosanoids (Bionaz et al., 2007; Sordillo et al., 2009). For these reasons, finding solutions to reduce
the incidence of health problems and the severity of inflammation during this physiological stage is important.

Nonsteroidal Antiinflammatory Drugs (NSAID) are routinely used against inflammation, exerting their action by inhibiting the cyclooxygenase enzymes (COX). We showed in the past that the administration of acetylsalicylic acid (ASA) to dairy cows reduced the negative consequences of inflammation and consequently led to greater milk yield, fertility, and metabolic status (Trevisi et al., 2005; Trevisi and Bertoni, 2008). In monogastrics, the omega-3 fatty acids (FA) also can play a role in the modulation of the inflammatory process by increasing the concentration of the antiinflammatory eicosanoids (Calder, 2006) or by influencing the activity of peroxisome proliferator-activated receptor reducing the nuclear factor-κB (NF-κB), the nuclear factor that triggers the transcription of proinflammatory genes (PPAR; Groeger et al., 2010).

We are not aware of experiments with transition cows dealing with simultaneous administration of ASA and omega-3 FA during late pregnancy, which in humans has been suggested to have positive synergistic effects (Engström et al., 2001). Our aim was to assess the possible synergistic action of omega-3 FA and low-dose ASA administered to dairy cows before calving on the natural systems leading to the resolution of inflammation in the transition period.

MATERIALS AND METHODS

This study complied with Italian and European rules on animal experimentation and ethics.

Barn Characteristics, Animals, and Treatments

The trial took place in the Università Cattolica del Sacro Cuore experimental barn (CERZOO) located in the Northern Italy (Piacenza) during the autumn-winter season and involved 26 multiparous Friesian dairy cows housed in free stalls with cubicles, and milked twice a day (12-h gap). Dry and lactating cows were fed 2 different total mixed rations (TMR) described in Table 1. Three homogeneous groups were formed according to body condition, calving period, production potential, parity, mammary gland health, and BW. The first group received a combination of omega-3 FA and ASA (n = 9; OMAS). Fish oil was supplemented from 21 d before the expected calving through the day before calving. The dose administered was 100 g/cow/d of product (Orovital Cod; Ascor Chimici Srl, Capocolle di Bertinoro, FC, Italy), corresponding to 75 g FA/cow/d. According to the composition of the product (Table 2), the cows received daily 12.0 g of omega-3 FA 4.6 g of eicosapentaenoic acid (EPA) and 6.0 g of docosahexaenoic acid (DHA); omega-6 FA were administered with the product at 1.6 g/d. The supplement was fed once each day in the morning immediately before the distribution of the TMR, and it was mixed with about 0.5 kg of the TMR fed to the fresh cows.

There was no possibility of competition among cows for ingesting the supplement. During the last 7 d of pregnancy, cows in OMAS received also a daily intramuscular injection of lysine acetylsalicylate (Lysal, Farmaceutici Gellini Srl, Peschiera Borromeo, MI, Italy) at a rate of 6.0 mg/kg BW of ASA (approximately 20% of the daily suggested dose for clinical illnesses). The second group (n = 9; OME) received the same omega-3 FA supplement as the OMAS group, but no ASA. The last group (n = 8; CTR) received, for the same 21-d period, hydrogenated palm oil (75 g/d) to balance the energy intake of the other 2 groups (Greenfat 3G, Or Sell Srl, Limidi di Soliera, Modena, Italy).

Clinical Checks

During the experiment, health status was checked every day through a general inspection and also monitored through a computerized system (Afimilk, S.A.E. Afikim, Kibbutz Afikim, Israel) based on the automatic recording of activity and milk production through a leg transponder. In addition, rectal temperature was measured the day after calving and twice a week from 14 d before to 14 d after calving. Cows were also submitted to a thorough gynecological examination at about 10 and 30 d in milk (DIM), or when deemed necessary if a pathological condition was apparent. Moreover, each cow underwent these assessments: i) body condition scoring, using a 5-point scale (Agricultural Development and Advisory, 1986), starting at ~35 d before the expected calving date and then every 14 d through 35 DIM; ii) milk yield and its conductivity, measured and recorded by the computer-controlled automated system at every milking; iii) milk samples at 7, 14, and 28 DIM, from the morning milking, to assess fat, protein, and lactose content (MilkoScan FT 120, Foss Electric, Hillerød, Denmark), and somatic cell count (SCC; Fossomatic 180, Foss Electric); iv) blood samples 9 mL for each tube, collected approximately (±3 d) at −28, −21 (pretreatment), −14, −10, −7, −3 (before calving), 1 (within 24 h from calving), 3, 7, 10, 14, 21, 28, and 35 (posttreatment) d relative to calving. Every sample was collected in the morning before feeding, from a jugular vein and in 2 vacuum tubes (Vacuette, Greiner Bio-One GmbH, Kremsmunster, Austria), 1 containing lithium-heparin as anticoagulant, and the other silicon (no anticoagulant). Lithium-heparin tubes were cooled immediately after collection in an ice-water bath until their arrival in the laboratory. A 75-μL aliquot of blood was taken from the heparin tube and centrifuged at 30,260 × g for 11 min. at room temperature to determine packed cell volume. The remaining sample was centrifuged
Table 1. Nutritional value of the diets fed as total mixed ration for dry and lactating cows

<table>
<thead>
<tr>
<th>Dietary component, % of DM</th>
<th>Dry cows</th>
<th>Lactating cows</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn silage</td>
<td>25.0</td>
<td>33.5</td>
</tr>
<tr>
<td>Corn grain ground</td>
<td></td>
<td>17.2</td>
</tr>
<tr>
<td>Alfafla hay</td>
<td></td>
<td>15.3</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>7.3</td>
<td>9.2</td>
</tr>
<tr>
<td>Cottonseed</td>
<td></td>
<td>7.7</td>
</tr>
<tr>
<td>Corn flakes</td>
<td></td>
<td>7.4</td>
</tr>
<tr>
<td>Grass hay</td>
<td>51.6</td>
<td>35</td>
</tr>
<tr>
<td>Sunflower seed</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>Mineral and vitamin supplement</td>
<td>1.0</td>
<td>2.1</td>
</tr>
<tr>
<td>Corn semolina glutinated</td>
<td></td>
<td>1.7</td>
</tr>
<tr>
<td>Wheat straw</td>
<td>15.3</td>
<td></td>
</tr>
</tbody>
</table>

Chemical composition

<table>
<thead>
<tr>
<th>NE, Meal/kg of DM</th>
<th>CP, % of DM</th>
<th>NDF, % of DM</th>
<th>Starch + sugar, % of DM</th>
<th>Ether extract, % of DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4</td>
<td>12.5</td>
<td>54.9</td>
<td>12.5</td>
<td>2.7</td>
</tr>
<tr>
<td>1.5</td>
<td>16.0</td>
<td>33.7</td>
<td>29.8</td>
<td>4.5</td>
</tr>
</tbody>
</table>

142.9% Ca<sub>P</sub>O<sub>4</sub>, 28.6% urea; 14.3% MgO; 7.1% NaCl; 7.1% mineral and vitamin supplement (15000 IU/kg vitamin A; 15000 IU/kg vitamin D; 7000 IU/kg vitamin E; 10 mg/kg Co; 70 mg/kg Mn; 500 mg/kg Cu; 23 mg/kg Se; 4000 mg/kg Zn).

2Water was added to each ration to reach the 52% of DM B.

Table 2. Amount of fatty acids supplemented daily to the cows fed omega-3 fatty acids and omega-3 fatty acids and acetylsalicylic acid from 21 d before calving to the last day of pregnancy

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Amount, g/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>Myristic acid</td>
</tr>
<tr>
<td>C16:0</td>
<td>Palmitic acid</td>
</tr>
<tr>
<td>C16:1ω-7</td>
<td>Palmitoleic acid</td>
</tr>
<tr>
<td>C18:0</td>
<td>Stearic acid</td>
</tr>
<tr>
<td>C18:1ω-9</td>
<td>Oleic acid</td>
</tr>
<tr>
<td>C18:2ω-6</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>C18:3ω-3</td>
<td>Linolenic acid</td>
</tr>
<tr>
<td>C18:4ω-3</td>
<td>Stearidonic acid</td>
</tr>
<tr>
<td>C20:0</td>
<td>Arachidic acid</td>
</tr>
<tr>
<td>C20:1ω-11</td>
<td>Icosenoic acid</td>
</tr>
<tr>
<td>C20:1ω-9</td>
<td>Eicosenoic acid</td>
</tr>
<tr>
<td>C20:2ω-6</td>
<td>Eicosatetraenoic acid</td>
</tr>
<tr>
<td>C20:4ω-6</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>C20:5ω-3</td>
<td>Eicosapentaenoic acid</td>
</tr>
<tr>
<td>C22:0</td>
<td>Behenic acid</td>
</tr>
<tr>
<td>C22:1ω-9</td>
<td>Erucic acid</td>
</tr>
<tr>
<td>C22:6ω-3</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>C24:1</td>
<td>Nervonic acid</td>
</tr>
<tr>
<td>Others</td>
<td>15.83</td>
</tr>
<tr>
<td>Total</td>
<td>74.51</td>
</tr>
<tr>
<td>o-3 total</td>
<td>12.02</td>
</tr>
<tr>
<td>o-6 total</td>
<td>1.51</td>
</tr>
</tbody>
</table>

at 3,520 × g for 16 min at 4°C; plasma samples were divided into 5 aliquots, stored at –20°C (n = 4) or –80°C (n = 1).

In accordance with the methods described by Bionaz et al. (2007), these samples were used to determine i) inflammatory response indexes: positive acute-phase proteins (+APP; haptoglobin, ceruloplasmin) and negative acute-phase proteins (–APP; albumin, cholesterol as lipoprotein index); ii) liver indices: total bilirubin, aspartate amino- tranferase (GOT), γ-glutamyl transferase (GGT), alkaline phosphatase, paraoxonase (PON), activated paraoxonase, sorbitol dehydrogenase (SDH); iii) energy metabolism indexes: glucose, NEFA, β-hydroxybutyric acid (BHB); iv) protein metabolism indexes: urea, creatinine; v) oxidative stress and related variables: total reactive oxygen metabolites (ROM), total nitric oxide metabolites, nitrites, nitrates (NO<sub>3</sub>), and thiol groups; vi) minerals (Ca, P, Mg, Na, K, Cl, and Zn); vii) vitamins: retinol (index of its carrier protein, Retinol Binding Protein; RBP), tocopherol, and β-carotene; viii) other variables (total proteins and globulins). Among the variables related to oxidative stress, we also assessed total antioxidants through the oxygen radical absorbance capacity assay. This method measures a fluorescent signal from a probe (fluorescein) that decreases in the presence of radical damage (Cao and Prior, 1999). The analysis was performed with a multidetection microplate reader equipped with a dual reagent injector (BioTek Synergy 2, Winooski, VT). All variables were assessed on the Litherein samples except for SDH (serum sample).

Data Handling and Statistical Analysis

If not specified, data in this paper are presented in the form of mean ± SD.

Repeated Measures ANOVA. Data were submitted to repeated measures variance analysis using a mixed model (MIXED procedure, SAS Inst. Inc., Cary, NC; Littell et al., 1998). Before analysis, the normality of distribution was verified for each parameter through skewness and kurtosis calculation according to the Shapiro test of SAS. When necessary, data were normalized through logarithmic, quadratic, inverse, or root-square transformations. The statistical model can be summarized as follows:

\[ Y_{ijklm} = \mu + G_i + T_k + GT_{ik} + B_{l(ijm)} + e_{ikl mn} \]

where \( Y_{ijklm} = \) mth observation of the lth cow \( B_i \) within the ith treatment \( G_i \), at the kth time to calving \( T_k \); \( \mu \) = total average; \( G_i \) = effect of the ith treatment (3 treatments: OME, OMAS, and CTR); \( T_k \) = effect of the kth time to calving (the number of levels being defined as a function of pregnancy phase and actual variable); \( GT_{ik} \) = effect
of the interaction between the $i$th treatment and the $k$th time to calving; $\beta_{ikm}$ = effect of the $i$th treatment on the $k$th cow within the $i$th treatment; $\epsilon_{ikm}$ = random effect or error.

The analysis was performed using 3 covariance structures: autoregressive, compound symmetry, and spatial power. These structures were ranked according to their AIC (Akaike’s Information Criterion; Akaike, 1974), choosing as better the lowest one (Littell et al., 1998). For each treatment, least squares means were computed, and preplanned pairwise comparisons (PDIF option, SAS) were performed when the $F$-test of one of the main factors (time, treatment, treatment × time) was significant at $P < 0.10$. Statistical significance was designated as $P < 0.05$; tendencies were declared at $P < 0.10$.

**One-Way ANOVA.** The differences among groups of some parameters (pregnancy length, number of artificial inseminations, days open) were evaluated through a 1-way ANOVA (GLM procedure, SAS) considering the group as a fixed factor. The layout of the statistical model can be summarized as follows:

$$Y_{ij} = \mu + G_i + e_{ij}$$

where $Y_{ij} = i$th observation of the group with $j$th parameter; $\mu = total average; G_i = effect of the i$th value ($i = group$); $e_{ij} = random effect or error$.

**Fertility Status Index Index Calculation.** The fertility status index (FSI) was calculated for each group according to Esslemont and Eddy (1977). This index is calculated for a group of cows and takes into account the number of pregnant cows on the total cows, the pregnant cows at first AI, the average number of AI per pregnancy, and the average days open.

**RESULTS**

The planned days of supplement administration differed slightly because of the calving date variability. The OME group received omega-3 FA for 19 ± 5 d before calving, and OMAS cows for 25 ± 5 d. The OMAS group received ASA for 8 ± 2 d and the CTR group received the administration of hydrogenated FA for 22 ± 7 d.

Evaluation of pregnancy length indicated that OME cows had shorter pregnancies with respect to the other groups (277 ± 6 d of pregnancy); CTR cows calved at 281 ± 6 d of pregnancy, and OMAS cows calved after 284 ± 5 d of pregnancy. The difference between OME and OMAS in days of pregnancy turned out statistically significant ($P < 0.02$).

**Animal Health**

Most of the cows involved in the experiment did not show signs of disease during the transition period. Some of them were affected by mild issues which did not require any kind of intervention (e.g., 1 d of high temperature or diarrhea). However, some cows were affected by severe pathologies immediately after calving. In the CTR group, 1 cow had lameness and 1 cow metritis. In the OME group, 1 cow had retained placenta (RP). In the OMAS group, 3 cows suffered from several diseases: 1 had RP, 1 had severe ketosis, and 1 had both RP and ketosis. The cows that suffered RP were treated with uterine oxytetracyclin tablets, and in 1 case with serotonin + ergometrine and an antiinflammatory drug. Cows with severe ketosis received jugular glucose injections for 2 to 3 d according to the severity of the pathology.

Regarding BCS, cows in all 3 groups had the typical postcalving decline. The decline was evident 1 wk before calving and continued through all the subsequent time points studied. From 7 d before calving to 35 DIM, cows in the OME group had a tendency ($P < 0.10$) to have a smaller decrease of BCS compared with CTR: 0.33 ± 0.19 vs. 0.57 ± 0.27 points of body condition. Cows in the OMAS group lost 0.51 ± 0.27 points (data not shown).

**Performance**

**Milk Yield and Quality.** Milk yield was not statistically different among groups: CTR cows produced 1158 ± 100 kg in the first 28 DIM, OME 1115 ± 258, and OMAS 1075 ± 115 kg. The assessed variables of milk quality did not have any statistical difference (data not shown).

**Fertility.** Five cows were culled due to infertility during the lactation after the experiment: 1 in CTR, 1 in OME, and 3 in the OMAS group. Days open in the CTR cows averaged 95 ± 46, with 1.7 ± 1.1 AI. For OME cows, the days open were 120 ± 47 and the number of AI was 2.3 ± 1.2. The OMAS group had 158 ± 55 d open and 3.3 ± 1.5 AI. The differences between CTR and OMAS were significant for days open ($P < 0.03$) and for number of AI ($P < 0.03$). The values of FSI index were 83.9 in CTR, 44.5 in OME, and –4.5 in OMAS.

**Blood Variables**

**Energy Metabolism.** In all groups glucose (Fig. 1) concentration peaked immediately after calving followed by a drop in the subsequent days. However, the glycemia in the OME group returned to precalving concentrations before than the other groups. At 7 DIM, the plasma concentration of glucose was 3.95 ± 0.24 mmol/L in OME, 3.42 ± 0.95 mmol/L in OMAS, and 3.52 ± 0.46 mmol/L in CTR ($P < 0.001$ OME vs. OMAS, and $P < 0.01$ OME vs. CTR).

Concentration of BHB peaked in OMAS at 7 DIM (1.63 ± 1.58 mmol/L; Fig. 1). In OME and CTR groups the peak occurred at 1 (0.81 ± 0.27 mmol/L) and 3 DIM...
Omega-3 and acetylsalicylic acid in transition dairy cow

(0.89 ± 0.62 mmol/L). At 7 DIM, BHB concentration was greater in OMAS than in the other 2 groups (P < 0.01 vs. OME, and P < 0.05 vs. CTR). At 10 DIM, the OMAS group tended (P < 0.10) to have greater concentration of BHB than OME (0.53 ± 0.14 mmol/L in OME vs. 1.02 ± 0.92 mmol/L in OMAS, but was similar to CTR (P > 0.10). A tendency for reduced BHB concentration was observed in OME vs. CTR (0.44 ± 0.10 vs. 0.68 ± 0.33 mmol/L at 21 DIM). The response of NEFA (data not shown; P > 0.10) was similar to that of BHB, with greater concentrations in OMAS and reduced in OME after calving. The peak of NEFA occurred in OMAS at 7 DIM (0.98 ± 0.50 mmol/L), and it was reached at 3 DIM in OME (0.68 ± 0.42 mmol/L) and CTR (0.73 ± 0.44 mmol/L).

Inflammatory Status. Concentration of haptoglobin (Fig. 2) was decreased (<0.2 g/L) in all the 3 groups until 7 d before calving. Three days before calving it started to increase in the OMAS group and reached a peak at 7 DIM (1.17 ± 0.90 g/L in OMAS; 0.29 ± 0.30 g/L in CTR, 0.40 ± 0.34 g/L in OME; P < 0.01 CTR vs. OMAS, and P < 0.05 OME vs. OMAS). The difference continued to be significant between CTR and OMAS at 10 and 14 DIM (P < 0.05). The CTR and OME groups reached a peak in concentration at 3 DIM (0.49 ± 0.43 g/L in CTR, 0.70 ± 0.38 g/L in OME), and subsequently decreased. At 21 DIM, the concentrations were similar among the 3 groups.

Cholesterol (considered an indirect index of lipoproteins; Fig. 3) concentration in OME was already greater at the beginning of the experiment (3.55 ± 0.71 mmol/L in OME, 2.79 ± 0.79 mmol/L in CTR and 2.69 ± 0.41 mmol/L in OMAS 28 d before calving; P < 0.01 vs. CTR and OMAS) than in the other 2 groups. Cholesterol concentration remained greater in OME for the whole period of the experiment. The concentration in CTR, similar to OMAS at 28 d before calving, decreased more markedly around calving and reached the greatest difference at 1 DIM (1.95 ± 0.46 mmol/L in OMAS, 1.70 ± 0.34 mmol/L in CTR; not significant, NS). At 10 DIM cows in OMAS tended to have reduced concentration compared with CTR (2.03 ± 0.65 mmol/L in OMAS, 2.36 ± 0.44 mmol/L in CTR; P < 0.10).

Albumin concentrations (Fig. 3) were similar before calving, and after parturition the increase was delayed in OMAS vs. CTR and OME: at 10 DIM the concentrations were 34.58 ± 3.17 g/L in OMAS, 36.86 ± 2.57 g/L in CTR, 36.58 ± 1.79 g/L in OME (P < 0.01).

Retinol (considered as index of retinol binding protein; Fig. 4) plasma concentration decreased close to calving in all the groups (P < 0.01). After parturition, the concentration of retinol increased rapidly in CTR and OME, but in OMAS the increase was slower. The greatest differences in retinol were reached at 10 DIM (33.79 ± 19.63 µg/100 mL in OMAS, 47.31 ± 15.22 µg/100 mL in OME, 51.56 ± 19.98 µg/100 mL in CTR;
Grossi et al. (2022) reported that the differences in concentration decreased at subsequent time points, and at 21 DIM they were similar.

Plasma PON concentrations (Fig. 4) were similar among groups before calving, whereas after calving it showed some differences: at 14 DIM the concentrations were 124.23 ± 24.72 U/mL in CTR, 126.43 ± 26.18 U/mL in OME, and 94.22 ± 28.85 U/mL in OMAS (P < 0.05 OMAS vs. CTR, and P < 0.01 OMAS vs. OME).

**Oxidative Stress.** The mean value of plasma total antioxidant concentration for all groups at 28 d before calving was 12.12 ± 2.51 mmol/L. At subsequent times it decreased until the day after calving (10.24 ± 2.60 mmol/L), and consequently, concentration increased during lactation (14.06 ± 2.38 mmol/L at 28 DIM). No significant differences were found among groups.

The plasma concentration of ROM decreased during the last month of pregnancy (P < 0.01), and at 7 DIM, the mean ROM concentration was 16.21 ± 2.41 H₂O₂/100 mL. As for total antioxidants, there was no statistical difference among groups for ROM.

**DISCUSSION**

**Effects of Omega-3 FA on the Inflammatory Status and on the Response to Inflammation**

Several published studies described improvements in inflammatory status in humans after the administration of omega-3 FA (Teitelbaum, 2001; Mori and Beilin, 2004; Calder, 2006; Clària et al., 2011). In fact, those FA are important molecules in the pathway of eicosanoid metabolism. The mechanism involves the COX, which initiates a cascade converting arachidonic acid (omega-6 FA) to a number of molecules that exert different actions in the inflammatory process. A prolonged administration of omega-3 FA causes their accumulation in tissues (Von Schacky et al., 1985). When an inflammatory event
occurs, also the omega-3 FA may be mobilized and partly replace the arachidonic acid; this causes the production of EPA with a lower proinflammatory potential (3-series PG and thromboxane; Mori and Beilin, 2004), and also other molecules involved in the resolution of inflammation (resolvins and protectins; Serhan and Savill, 2005). Groeger et al. (2010) demonstrated the formation of electrophilic oxo-derivatives (EFOX) from EPA and DHA in activated macrophages, through COX-2. This mechanism is enhanced with the modulation of ASA. The EFOX exert an antiinflammatory effect through an agonist action on the gene expression of the PPARγ which inhibits the NF-κB when bound to omega-3 FA. As a consequence it also inhibits the expression of genes which encode proinflammatory proteins (e.g., cytokines, nitric oxide synthase, COX).

The lack of response between OME and CTR as it relates to APP suggest that the administration of omega-3 FA alone in late pregnancy did not exert any significant effect on the inflammatory status of cows around calving. This response does not agree with a previous experiment (Trevisi et al., 2011), in which a better inflammatory status after calving was observed with supplementation of omega-3 FA. However, the discrepancy could be partly attributed to the longer period of omega-3 FA administration (pre- and postcalving) or to the contemporaneous administration of vitamin E in Trevisi et al. (2011).

**Effects of Omega-3 FA in Combination with ASA on the Inflammatory Status**

Acetylsalicylic acid is widely used for its potent antiinflammatory effect exerted through the inhibition of both COX-1 and COX-2 (Vane, 2003). This action is also effective at low dose in murine models (Cyrus, 2002) and has been useful in preventing inflammation in patients affected with cardiovascular diseases (Patrono et al., 2005). The antiinflammatory effect of ASA was confirmed using small animals, humans, and dairy cows (Vane, 1971; Gingerich et al., 1975; Vane, 2003; Bertoni et al., 2004). Trevisi and Bertoni (2008) administered ASA to dairy cows in the postparturient period, which resulted in positive effects on milk yield, fertility, health conditions, and inflammatory status. Serhan et al. (2002) demonstrated in murine models the effectiveness of the combination of low-dose ASA and omega-3 FA to enhance the resolution of inflammation through the production of resolvins. In humans, the combination of omega-3 FA and ASA has resulted in some improvements of the eicosanoid pattern (e.g., reduction in the production of the proinflammatory types; Engström et al., 2001).

Our aim was to verify the possible synergistic action of omega-3 FA together with a low dose of ASA as a means to reinforce the natural systems of inflammation resolution during the last days of pregnancy of dairy cows. The transition period is characterized by inflammatory processes, which if not properly controlled, could lead to disease (Bionaz et al., 2007; Sordillo et al., 2009; Trevisi et al., 2009). Our data concerning the inflammatory status demonstrate that all the groups experienced an inflammatory condition around calving, as demonstrated by the raise of the +APP (e.g., haptoglobin) and in agreement with Bertoni et al. (2008) and Trevisi et al. (2010a and b). Nevertheless, the concentrations of haptoglobin postcalving indicated that cows in OMAS were more affected by inflammatory conditions. This suggests that all OMAS cows (with or without clinical symptoms) suffered more severe and prolonged negative consequences to inflammation in comparison with CTR cows. In fact, the OMAS cows showed an earlier increasing trend of plasma haptoglobin than the other groups. The worse inflammatory status in OMAS cows was confirmed by the lower plasma concentrations of the liver proteins (e.g., +APP like albumins, lipoproteins, PON, RBP) likely due to the downregulation of their genes during the acute phase reaction (Schreiber et al., 1986).

The OMAS group showed during the first month of lactation a slower recovery of the plasma concentration of +APP (cholesterol, albumin, retinol), which are correlated to worse performance in dairy cows. Indeed, the cows in this condition showed a reduced milk yield and DMI in the first month of lactation, as well as the worst reproductive indices, confirming the findings of Bertoni et al. (2008) and of Trevisi et al. (2010a).

The marked inflammatory processes observed in the OMAS group are in agreement with the greater incidence of clinical disease (33% of cows), mainly RP and ketosis, and consequently drug treatments (33% of cows needed antibiotic and/or antiinflammatory treatments in the first week of lactation). This seems not a direct effect of the ASA treatment, but it could be a consequence of the metabolic upsets due to the lower synthesis of PGF₂α as discussed below. The cows recovered from the health problems reported at calving, as confirmed by the good milk yield at the end of the first month of lactation.

**Effects of Treatments on Energy Metabolism**

One of the most important events during the transition from pregnancy to lactation is the inadequate nutrient intake that is partly overcome by the mobilization of body fat and muscle reserves (homeoretic mechanism). In addition, the immunosuppression status often observed in late pregnancy (Kehrl et al., 1989; Lacetera et al., 2005) may be worsened by the oxidative stress which occurs after calving (Sordillo and Aitken, 2009). These challenging conditions explain the greater frequency of metabolic and infectious diseases during the transition
period and the consequent worsening of negative energy balance (NEB; Drackley, 1999). The OMAS cows had more health problems, and in fact faced severe negative consequences due to inflammation which partly determined their energy balance status. Inflammation could have decreased feed intake and, hence, energy efficiency as previously suggested by Trevisi et al. (2010a). The severe NEB in the OMAS group was confirmed by the greater concentrations of BHB (vs. CTR and OME) after calving and by the 2 cases of clinical ketosis recorded, as well as the numerically lower milk yield with the similar reduction of BCS.

With respect to CTR cows receiving hydrogenated palm oil, the administration of omega-3 FA (OME group) may have exerted some positive effects on energy metabolism as suggested by the smaller decline of BCS and a similar milk yield in OME group. The decreased lipomobilization in OME cows is confirmed by the reduced concentration of BHB and the greater of glucose compared with CTR and to OMAS cows. A better postcalving energy status in cows fed a diet enriched in omega-3 FA was previously observed by Ballou et al. (2009) and by Trevisi et al. (2011). Conversely, Mattos et al. (2004) found decreased plasma concentrations of glucose and greater BHB in cows fed omega-3 FA vs. cows fed olive oil, whereas no differences were observed in NEFA. However, in this last experiment, fish oil was administered in a large amount and in a non-rumen-protected form, likely causing a strong reduction in DMI and several other problems reported by the authors.

**Effects of Treatments on Fertility**

Our data show that CTR cows had optimal reproductive performance (days open < 100 and AI < 2), confirmed also by the high concentration of FSI index, and, in fact, better than the ideal value (80 points) suggested by Esslemont and Eddy (1977). Cows in OME had acceptable values of days open and AI, better than the average values found in the area (Piacenza province) at the time of the experiment (179 d open and 2.8 AI; Associazione Italiana Allevatori, 2010). The worse fertility performances found in the OMAS group, in comparison with the other groups, may be attributed to the negative consequences of inflammation directly exerted by its mediators (e.g., cytokines, eicosanoids), as previously shown in humans (Ricciotti and FitzGerald, 2011) or indirectly by worsening the energy balance, as previously suggested by Bertoni et al. (2009).

**Possible Implications in Prostaglandin Metabolism**

The oral administration of omega-3 FA in mammals can alter the synthesis and the metabolism of prostaglandins, mainly due to the decrease in concentration of arachidonic acid in phospholipids (Simopoulos, 2008). An altered PG pattern affects several aspects connected to reproduction from calving time (Abayasekara and Wathes, 1999). Also the ASA is reported to inhibit the PGF$_{2\alpha}$ production, leading to the occurrence of some problems related to parturition (decreased uterus motility and delayed fetal membranes expulsion). This molecule exerts an inhibitory effect on COX (Vane, 2003), causing a decrease in the production of PGF$_{2\alpha}$. Several studies demonstrated the relationship between a reduced plasma concentration of PGF$_{2\alpha}$ and complications of aspects associated with labor and uterine motility. In particular, a reduced uterine activity was shown in rats and humans after the administration of NSAID (Csapo et al., 1973; Besinger et al., 1991). This action is exerted through the inhibition of COX, the reduction of PG, and consequently through the impairment of the regulation of vessel tone during fetal life and circulatory disorders at birth (Grella and Zanor, 1978). Currently, NSAID are used in humans to delay preterm labor (Loudon et al., 2003; Olson and Ammann, 2007; Livshits and Seidman, 2010).

In dairy cows, the above mechanism has been connected to RP (Chassagne and Barnouin, 1992; Takagi et al., 2002) and to an inadequate uterine involution (Lindell et al., 1982; Thompson et al., 1987). Although we did not determine the concentration of PGF$_{2\alpha}$ metabolites, it is possible to speculate that the administration of ASA exerted a depressing effect on the production of PGF$_{2\alpha}$, causing its decreased plasma concentration before calving. Such an effect might justify the calving delay of OMAS cows by 3 d on average in comparison with CTR cows and by 7 d in comparison with OME, confirming the supposed inhibitory effect of ASA on the release of PGF$_{2\alpha}$, even if administered at low-doses. Consequently, a reduced concentration of PGF$_{2\alpha}$ may cause a reduction of myometrial contraction, as well as greater occurrence of RP (and the related inflammation), suffered by the 25% of OMAS cows.

The administration of omega-3 FA alone (OME group) did not exert any effect on the delivery of fetal membranes; thus, it is reasonable to assume that it did not markedly affect the metabolism of PGF$_{2\alpha}$ around calving. In addition, the shorter pregnancy length in OME group suggests a nondelaying effect of calving of the omega-3 FA, in contrast to the findings of Baguma-Nibasheka et al. (1999), which showed in ewes as 0.6 g/kg BW/d of infused omega-3 FA delayed preterm induced labor through a reduced synthesis of 2-series prostaglandin. The reduction of PGF$_{2\alpha}$ has been reported by Petit et al. (2002) and Petit and Twagiramungu (2006), who reported an improvement in the gestation rate of lactating dairy cows fed flaxseed (rich in α-linolenic acid and not in EPA and DHA) during lactation, partly attributed by the authors to a reduced production of PGF$_{2\alpha}$. Differently
from our experiment, these studies administered a greater amount of the omega-3 FA (around 30-fold more than our supplement) and for a longer period. Therefore, the effect of omega-3 FA (type, dose, period, and length of treatment) on PG metabolism needs further investigation.

**Conclusion.** The attempt to attenuate inflammatory processes at calving, through the precalving use of low-dose NSAID in combination with omega-3 FA, did not exert any positive effect. In fact, such a treatment caused a lengthening of pregnancy, an increase in the frequency of retained placenta and a worsening of the inflammatory status and of the energy balance in the first 2 wk of lactation. On the contrary, the results of this experiment confirm the usefulness of an omega-3 FA administration in the peripartum period of dairy cows to improve energy metabolism immediately after calving. Our results also demonstrate that the effects of an omega-3 FA administration only in late pregnancy on the inflammatory status are weaker than when administered during the whole transition period. The cause of the better energy status in the cows fed omega-3 FA is not easily explained in the absence of a significantly better inflammatory status. Some slight improvements in the inflammatory response (e.g., smaller reduction at calving followed by quicker rise of albumin and vitamin A-RBP) might have contributed to this outcome. Major improvements can be obtained with a longer period of omega-3 FA administration, also in combination with vitamin E to avoid oxidative problems. Our results suggest that efforts to mitigate the negative consequences of inflammation at calving in dairy cows are important. We suggest that the administration of omega-3 FA during the whole transition period, in combination with antioxidants, is a suitable strategy. Even low-doses of NSAID should be administered starting 24 h after calving or later, after the delivery of the placenta, to avoid the possible interference in PG metabolism, which is of major importance during these phases.

**LITERATURE CITED**


