



# Effect of dietary supplementation with long-chain n-3 fatty acids during late gestation and early lactation on mare and foal plasma fatty acid composition, milk fatty acid composition, and mare reproductive variables



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## ABSTRACT

The effects of dietary marine-derived n-3 fatty acids (FA) on mare milk and mare and foal plasma FA, postpartum ovarian follicular growth and prostaglandin concentrations were evaluated. Sixty days prior to expected foaling dates, mares were assigned to one of three diets: corn oil (CORN,  $n = 6$ ), docosahexaenoic acid (DHA) diet (D; 12.64 g/d,  $n = 7$ ), or eicosapentaenoic acid (EPA) and DHA (ED; 8.84 g EPA, 10.43 g DHA/d,  $n = 7$ ). Milk and plasma were collected for FA analysis. Follicular data were recorded through the first postpartum ovulation. Post-ovulation serial blood samples were evaluated for prostaglandin  $F_{2\alpha}$  metabolite (PGFM). Supplementation with DHA, or DHA plus EPA resulted in lower linoleic acid and greater EPA and DHA in mare plasma ( $P < 0.05$ ), greater arachidonic acid and DHA, or EPA and DHA in milk ( $P < 0.05$ ), and greater DHA, or EPA and DHA in foal plasma ( $P < 0.05$ ). Days to the first postpartum ovulation was greater ( $P < 0.01$ ) in ED ( $22.5 \pm 2.1$ ) compared to CORN ( $12.5 \pm 2.3$ ) and D ( $13.3 \pm 2.3$ ) groups. Follicular retention ( $\geq 35$  mm) prior to ovulation was longer ( $P < 0.05$ ) for ED ( $12.7 \pm 1.9$  d) compared to CORN ( $6.3 \pm 2.0$  d) or D ( $6.0 \pm 2.0$  d) groups. Treatment did not affect PGFM concentrations. Maternal EPA and DHA supplementation beginning in late gestation altered the FA profile of milk and mare and foal plasma, and may result in delayed ovulation in the early postpartum period.

## 1. Introduction

Fat supplementation of horse diets is a common practice, particularly with older animals and those participating in strenuous activity. Although the beneficial effects of dietary fat are well-documented (Rich, 2004), there is interest in the precise role or contribution of individual fatty acids (FA) on horse health and performance. The two classes of essential polyunsaturated FA (PUFA) are of the n-6 and n-3 type. The n-6 FA, linoleic acid (LA), serves as the precursor for arachidonic acid (ARA), from which biologically active eicosanoids like the prostaglandin (PG) subfamily are derived. The n-3 FA,  $\alpha$ -linolenic acid (ALA), is converted to eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Most commercial concentrates for horses include plant-based fat sources, which have relatively greater concentrations of n-6 FA, thereby there is a greater dietary ratio of n-6 to n-3 PUFA in horses fed larger

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amounts of these concentrates.

Modulation of dietary FA can alter the FA composition of plasma and milk. Plasma EPA and DHA concentrations were not changed as a result of feeding horses flaxseed (Siciliano et al., 2003), but were increased in plasma (Siciliano et al., 2003; Kruglik et al., 2005; O'Connor et al., 2007) and milk (Kruglik et al., 2005; Stelzleni et al., 2006) in response to fish oil dietary supplementation. Reproductive variables are also modified by inclusion of long chain PUFA in the diet. Omega-3 supplementation affected ovarian and uterine function in ruminants (Abayasekara and Wathes, 1999; Mattos et al., 2000), and improved litter size in gilts and sows (Webel et al., 2007). In addition, both serum PG metabolites (Mahla et al., 2017; Verma et al., 2018) and cultured endometrial PG (Chaudhari et al., 2018) were less following fish oil supplementation in does. Likewise, endometrial gene expression for three enzymes associated with PG biosynthesis in sows was altered following dietary supplementation with flaxseed oil (Gokuldas et al., 2018).

In comparison to other species, other than the greater plasma concentrations of n-3 FA, little is known about the effects of supplementing pregnant or lactating mares with marine-based n-3 FA, specifically diets with greater concentrations of DHA alone or DHA combined with EPA. One of the objectives of the current study, therefore, was to test the hypothesis that maternal dietary supplementation with DHA or DHA and EPA would result in an increase in both mare plasma and milk n-3 FA concentrations, and as a consequence, there would be greater foal plasma FA at birth and during early lactation. Furthermore, postpartum ovulation data were obtained to test the hypothesis that supplementation would alter ovarian follicular dynamics in early postpartum mares. A final objective was to quantify the PGF<sub>2α</sub> metabolite (PGFM) in response to an oxytocin (OXY) administration during late diestrus to determine if DHA or EPA supplementation would attenuate the expected increase in PGFM concentrations.

## 2. Materials and methods

### 2.1. Animals and experimental design

This study was reviewed and approved by the Kansas State University Animal Care and Use Committee before the study began. Gravid Quarter Horse-type mares ( $n = 20$ ), ages 4–19 years with an average starting BW of  $539.10 \pm 9.86$  kg (mean  $\pm$  SE), along with their foals, were used in this study. Mares were housed throughout the study at the Kansas State University (KSU) Horse Unit in Manhattan, KS in one of two 2.0 ha pastures with minimal forage. All pregnant mares were housed together in one pasture, and subsequently moved to a second pasture after foaling. Brome hay was group fed at a daily rate of 9.1 kg/mare. Mares had *ad libitum* access to fresh water and a salt block, and all mares were fed a textured concentrate at a rate of either 3.6 kg/mare (pre-partum) or 5.4 kg/mare (postpartum) to meet NRC recommendations for diets of mares (2007). The concentrate was supplied by the KSU Feed Mill. Mares were fed the same basal diet at least 2 months prior to starting the experiment.

Mares were grouped by age, parity, and expected foaling date. The groups were considered blocks and the three dietary treatments were assigned to mares within the blocks. The specific treatments were a diet: supplemented with corn oil (CORN), DHA (D), or EPA and DHA (ED). The CORN group ( $n = 6$ ) was fed the base concentrate with 113.4 g of corn oil added to the base diet at the time of feeding. The D group ( $n = 7$ ) was fed the concentrate and there was DHA in amounts of 12.64 g each day added to the diet at the time of feeding. The ED group ( $n = 7$ ) was fed the concentrate with an EPA/DHA supplement providing 8.84 g EPA and 10.43 g DHA each day. This amount was chosen based on results from a previous study (Kruglik et al., 2005) in which mare plasma alterations in EPA and DHA occurred when there was dietary inclusion of a similar product at similar amounts of supplementation. Treatment supplements were derived from fish oil or algae and were early prototypical formulations of n-3 powder supplements for horses that were in development by JBS United, Inc. of Sheridan, IN. Although the D supplement was enriched in DHA, daily supplementations contained EPA in amounts of 0.60 g. A supplement with EPA alone was not available when the experiment was initiated. The n-3 dietary supplements were formulated to be isocaloric, and corn oil was added to the diet in amounts that also resulted in an isocaloric diet. In addition to a relatively greater palatability, corn oil was included as the control supplement based on its negligible n-3 content. Supplementation began 60 days prior to each mare's expected foaling date and ended during the second postpartum estrous period. The chemical analysis and fatty acid composition of feedstuffs and supplements are presented in Table 1.

The concentrate and supplements were weighed daily and divided into two equal amounts for feeding at the 0800 and 1600 h. Prior to parturition, mares were fed in individual pens or haltered and tied to ensure they received the specified dietary treatment. After foaling, mares were fed in individual pens and care was taken to ensure foals were not allowed access to the concentrate. During the pre-partum period, mares were monitored daily for signs of impending parturition. Physical characteristics and mammary secretions were evaluated and based on observed changes, mares were placed in stalls when parturition was considered to be imminent and monitored until foaling occurred.

### 2.2. Blood and milk sample collection

A 10 mL blood sample was collected using the jugular venipuncture technique into heparinized tubes (Vacutainer) from pregnant mares at the beginning of the study and every 2 wk until parturition. Blood was also collected from both the mare and foal at parturition, 12 h postpartum, and weekly intervals until the end of treatment when a final sample was obtained. The foal sample collected at birth was taken prior to the first suckling bout. Following blood collection, samples were centrifuged at  $2500 \times g$  for 20 min and plasma was harvested and frozen at  $-20^\circ\text{C}$  until analyzed for the FA content.

Samples of colostrum were obtained from mares at parturition prior to foals suckling for the first time, and again at 12 h postpartum. Additional milk samples were collected weekly until the end of the experiment. Between 15 and 20 total mL of milk were

**Table 1**

Chemical analysis<sup>a</sup> and fatty acid composition (expressed as % of total fat) of feedstuffs, corn oil, and a docosahexaenoic acid (DHA) supplement, or an eicosapentaenoic acid (EPA)/DHA supplement fed to mares during late gestation and early lactation.

Item <sup>b</sup>	Feedstuffs		Supplements		
	Grain mix	Brome hay	Corn oil	DHA	EPA / DHA
DM, %	89.19	91.47	100.00	95.92	93.05
Crude Protein, %	18.37	4.82	0.00	12.45	11.42
ADF, %	8.50	43.07	0.00	24.03	4.67
NDF, %	18.65	72.49	0.00	8.89	8.90
Fat, %	5.43	2.21	99.90	52.50	16.86
Ash, %	6.64	7.83	0.00	9.45	2.25
Ca, %	1.68	0.41	0.00	0.01	0.39
P, %	0.84	0.07	0.00	0.11	0.12
K, %	0.99	0.88	0.00	0.62	0.80
DE (Mcal/lb)	1.64	0.91	4.09	1.77	1.56
<b>Total n-6:</b>	<b>29.08</b>	<b>8.81</b>	<b>46.32</b>	<b>0.89</b>	<b>3.40</b>
Linoleic acid	29.08	8.81	46.30	0.07	2.39
Arachidonic acid	0.00	0.00	0.00	0.82	1.01
<b>Total n-3:</b>	<b>2.57</b>	<b>8.19</b>	<b>0.91</b>	<b>43.55</b>	<b>36.96</b>
Alpha-linolenic acid	2.55	8.12	0.91	0.00	2.33
EPA	0.01	0.03	0.00	1.89	11.56
Docosapentaenoic acid	0.00	0.00	0.00	0.42	2.12
DHA	0.00	0.00	0.01	39.88	13.62
n-6:n-3 ratio	11.32	1.08	50.90	0.02	0.09

<sup>a</sup> Calculated on a DM basis by SDK Laboratories, Hutchinson, KS.

<sup>b</sup> Values listed as means.

manually collected from both teats at each sampling time and individually frozen at  $-20^{\circ}\text{C}$ . Only colostrum samples were analyzed for IgG(T) concentration, whereas all milk samples were analyzed for FA content. Blood samples collected from each foal at birth as well as at 12 h and 28 days postpartum were also analyzed for IgG(T) concentration. Equine IgG(T) was used as an estimate of humoral immunity due to its prevalence in both plasma and milk as an abundant isoform of IgG (Sheoran et al., 2000). A plasma pre-suckling sample was not obtained from one foal in the ED and CORN group due to an unexpected foaling. Consequently, the data from these samples were not included in the parturition plasma and IgG(T) statistical analysis. All plasma and milk samples collected on a weekly or biweekly basis were procured at approximately the same time of day so that potential effects of circadian rhythmicity on this variable could be minimized (Orme et al., 1994).

At 1300 h, on day 15 following the first postpartum ovulation, 14 ga catheters were inserted into the jugular vein of each mare. A 10 mL blood sample was collected in serum tubes (Vacutainer) every 15 min for 3 h beginning 30 min after the insertion of the catheter. Baseline samples were collected for the first 60 min. Immediately following the 60 min sample, 20 IU of OXY was administered IV to stimulate a PGF<sub>2α</sub> release. Samples were then collected for 120 min following the OXY administration. Blood samples were allowed to clot at room temperature for 30 min and then centrifuged at 2500 x g for 20 min. Serum was harvested and frozen at  $-20^{\circ}\text{C}$  until analyzed for PGFM. During the sampling period, mares and foals were housed in a 3 × 9 m outdoor enclosure with access to fresh water, and foals were not prevented from suckling.

### 2.3. Reproductive measurements

Beginning on day 4 postpartum, follicular characteristics were monitored daily using transrectal ultrasonography. The diameter of all follicles measuring more than 20 mm on each ovary were recorded. Ovaries were observed using ultrasonography daily until the presence of a corpus luteum could be confirmed following the first postpartum ovulation. Beginning on day 12 post-ovulation, ovaries were again assessed using ultrasonography daily through day 15 post-ovulation. If at least one follicle was of a diameter of  $\geq 35$  mm on day 15, the experiment ended for that mare on day 15 following the OXY administration and blood sample collections. If the largest follicle was of a diameter of  $< 35$  mm, daily ultrasonic assessments continued until a 35 mm follicle was detected and at that time the inclusion of these mares in the experiment ended. This end point was required due to breeding that occurred at the time of the second estrous cycle for some of the mares. All ultrasonic measurements were performed by the same technician. Ancillary variables including gestation length, total time on the study, and time to placental expulsion were also recorded.

### 2.4. Milk and plasma analyses

The FA composition of the plasma and milk samples was analyzed using gas chromatography procedures as subsequently described. Samples (500  $\mu\text{L}$  of plasma or 200  $\mu\text{L}$  of milk) were added to 10 mL screw cap tubes and freeze dried. Benzene (1 mL) containing an internal standard (methyl-C13, 400  $\mu\text{g}/\text{mL}$  for plasma and 1000  $\mu\text{g}/\text{mL}$  for milk) and 4 mL of 10% BF<sub>3</sub>-Methanol reagent (Supelco, Inc., Bellefonte, PA) were added. Tubes were capped, vigorously mixed, and incubated at  $60^{\circ}\text{C}$  for 60 min. After

cooling, 4 mL of water and 1 mL of hexane were added and tubes were again mixed vigorously. The benzene/hexane layer was separated by centrifuging at 1000 x g for 5 min and then transferred to vials for long-chain fatty acid (LCFA) analysis.

Samples were analyzed for FA methyl esters using a Hewlett-Packard 5890 gas chromatograph with a SP-2560 capillary column (Supelco, Inc., Bellefonte, PA). Injection port and detector temperatures were 250 °C with a flow rate of 1 mL/min and a split ratio of 100:1. Initial oven temperature was 140 °C and there was an increase at 2 °C/min to 200 °C, and the temperature was increased 4 °C/min to 245 °C and this temperature was maintained for 17 min. Although chromatographic analysis resulted in the isolation and quantification of numerous FA, data were analyzed and results are reported for the following FA of particular interest: LA, ARA, ALA, EPA, and DHA.

Foal plasma and milk IgG(T) were measured by a commercially available ELISA kit (Bethyl Laboratories, Inc., Montgomery, TX). The detection limit was 15.6 ng/mL and the inter- and intra-assay coefficients of variation were 3.9% and 2.2%, respectively. A commercial ELISA kit (Neogen Corporation, Lexington, KY) was also utilized to quantify plasma PGFM. Samples were first extracted according to the manufacturer's directions using the following protocol. In brief, a C18 Sep-Pak column was preconditioned with 2 mL of methanol, followed by 2 mL of water. Then 0.2 mL of methanol was added to 1 mL of sample plasma. This combined mixture was added to the column and washed with 2 mL of 15% methanol in water, followed by 2 mL of petroleum ether. Then, 2 mL of methyl formate was added to the column. The liquid filtered through the column was dried using nitrogen gas and the residue was dissolved in 1 mL of diluted extraction buffer provided as a part of the kit reagents. This final extracted product was then quantified using the ELISA. The sensitivity concentration of the assay was 20.0 pg / mL, and the inter- and intra-assay coefficients of variation were 6.4% and 2.8%, respectively.

### 2.5. Statistical analysis

All data were analyzed using an ANOVA and the MIXED procedure of SAS (Version 9.01, SAS Inst. Inc., Cary, NC). Data measured at one time point were analyzed as a randomized incomplete block (incomplete because all three treatments did not appear in every block) design with treatment as a fixed effect and block as a random effect. Data measured over time were analyzed as a randomized incomplete block design with treatment and time as fixed effects and block and horse as random effects using PROC MIXED for repeated measures. Blocks were groups of mares. The groups were based on two age classes (< 10 yr or ≥ 10 yr), two parity classes (primiparous or multiparous), and three expected foaling date classes (March, early to mid-April, or late-April to May) for a potential of  $2 \times 2 \times 3 = 12$  total unique blocks. Type 3 tests of fixed effects were used to determine differences due to time, treatment, and the time by treatment interaction for FA, IgG, and PGFM concentrations. Data are expressed as LS means  $\pm$  SE unless otherwise noted, and differences were considered significant at  $P < 0.05$ .

## 3. Results

### 3.1. Plasma FA analysis

By 2 weeks after the start of supplementation, pre-partum mare plasma LA was less ( $P < 0.05$ ) in D and ED compared with the corn oil group (Table 2), with differences continuing throughout parturition (Table 2). Plasma ALA concentrations were similar among groups (Table 2). Plasma ARA concentrations were greater ( $P < 0.05$ ) in the D compared to CORN group beginning at wk 2, and were greater ( $P < 0.05$ ) than that of both the CORN and ED groups by wk 6 and remained greater in the D group for the duration of the study (Table 2). The  $P$ -values for effect of time, treatment, and time by treatment interaction on LA, ALA and ARA concentrations are listed in Table 2. Concentrations of EPA remained at baseline for the CORN group, however, were greater in D ( $P < 0.05$ ) and ED ( $P < 0.01$ ) groups from wk 2 until parturition (Fig. 1). During the postpartum period, EPA concentrations remained greater ( $P < 0.01$ ) in the ED compared to both D and CORN groups. By wk 2, plasma concentrations of DHA were greater ( $P < 0.01$ ) in D and ED compared to the CORN group, and remained greater throughout the study (Fig. 1). There were effects ( $P < 0.01$ ) of time, treatment, and the time by treatment interaction on plasma EPA and DHA. For both D and ED groups, plasma LA, ARA, EPA, and DHA peaked around the time of parturition.

In foal plasma, there was no effect of maternal dietary treatment on concentrations of LA or ALA at any time point for which there were determinations (Table 3). Foal LA and ALA concentrations were least ( $P < 0.01$ ) at birth compared to all other time points. Plasma ARA concentration of foals in the CORN group was greater ( $P < 0.05$ ) at birth compared to the D and ED groups. On day 7, 14, and 21, D foals, however, had greater ( $P < 0.05$ ) ARA concentrations compared with foals in both the CORN and ED groups (Table 3). The  $P$ -values for effect of time, treatment, and the time by treatment interaction on the concentrations of LA, ALA, and ARA analysis are listed in Table 3. At birth, foal plasma EPA was greater ( $P < 0.01$ ) in the foals of the ED group compared with those in the CORN group, and remained greater for the first 21 days compared to foals in both the CORN and D groups (Fig. 2). Even though there were no treatment differences at birth, plasma DHA concentrations were greater ( $P < 0.01$ ) in the foals of the ED and D group compared to CORN group between day 7 and 21, and concentrations remained greater ( $P < 0.01$ ) in the D compared to ED group at 14 and 21 days after birth (Fig. 2). There were effects ( $P < 0.01$ ) of time, treatment, and time by treatment interaction on plasma EPA and DHA concentrations of foals.

### 3.2. Milk FA analysis

Similar to the foal plasma, there were no significant treatment effects on milk LA or ALA concentrations at any of the time points

**Table 2**

Mare plasma linoleic acid (LA), alpha-linolenic acid (ALA), and arachidonic acid (ARA) concentrations following supplementation with corn oil (CORN), a docosahexaenoic acid supplement (D), or an eicosapentaenoic and docosahexaenoic acid supplement (ED) during late gestation and the early postpartum period<sup>1</sup>.

Fatty acid, µg/mL	Treatment <sup>2</sup>			PooledSEM	P-value		
	CORN	D	ED		Trt	Time	T x T <sup>4</sup>
<b>LA</b>					< 0.01	< 0.01	< 0.01
Start	723.55	741.66	653.46	37.74			
Wk 2	732.39 <sup>a</sup>	617.61 <sup>b</sup>	522.42 <sup>b</sup>	37.74			
Wk 6	817.01 <sup>a</sup>	639.83 <sup>b</sup>	547.07 <sup>b</sup>	37.74			
Parturition	917.61 <sup>a</sup>	732.89 <sup>b</sup>	669.84 <sup>b</sup>	37.74			
Day 21 postpartum	689.35	581.76	581.88	37.74			
End <sup>3</sup>	661.58	577.38	585.69	38.40			
<b>ALA</b>					0.15	< 0.01	0.95
Start	53.85	51.86	45.82	6.49			
Wk 2	30.25	25.92	22.34	6.49			
Wk 6	45.49	48.30	33.37	6.49			
Parturition	41.84	34.18	25.01	6.49			
Day 21 postpartum	35.43	28.55	23.23	6.49			
End	24.10	26.14	27.12	6.66			
<b>ARA</b>					< 0.01	< 0.01	0.13
Start	17.41	18.80	16.71	1.44			
Wk 2	16.68 <sup>a</sup>	21.37 <sup>b</sup>	17.76 <sup>a,b</sup>	1.48			
Wk 6	21.39 <sup>a</sup>	30.10 <sup>b</sup>	21.77 <sup>a</sup>	1.48			
Parturition	22.93 <sup>a</sup>	30.21 <sup>b</sup>	25.23 <sup>a</sup>	1.48			
Day 21 postpartum	12.89 <sup>a</sup>	21.04 <sup>b</sup>	16.80 <sup>a</sup>	1.48			
End	11.22 <sup>a</sup>	18.23 <sup>b</sup>	13.14 <sup>a</sup>	1.51			

<sup>a,b</sup>Within a row, means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> Samples collected 60 days prior to expected foaling date (start), wk 2 and 6 following start of supplementation, at parturition, day 21 postpartum, and when a 35-mm follicle was first measured at the start of the second postpartum estrous period (end).

<sup>2</sup> LS means.

<sup>3</sup> All means from the start of the experiment through day 21 based on  $n = 6$  for CORN and  $n = 7$  for D and ED mares. End means for D mares based on  $n = 6$ .

<sup>4</sup> Treatment x Time interaction.

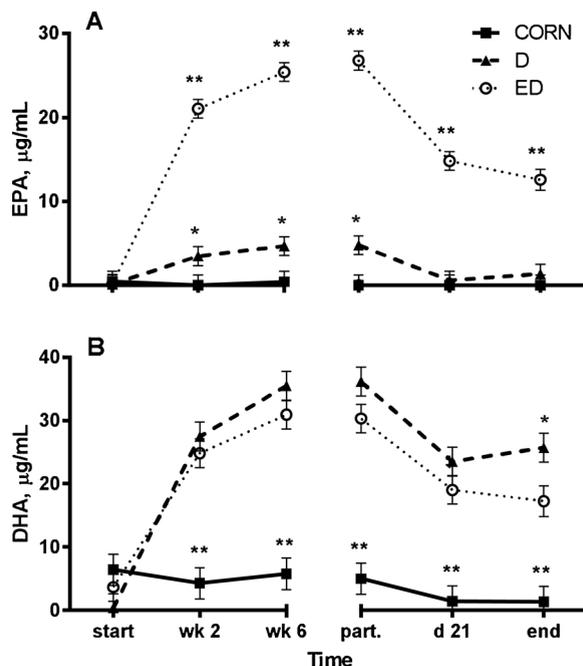
(Table 4), however, there was an effect of time. At 12 h postpartum, milk LA concentration was greater ( $P < 0.01$ ) for all groups compared to concentrations at parturition, 14, and 21 days postpartum. Milk ALA concentrations were greater after parturition, and ( $P < 0.05$ ) between 7 and 21 days postpartum compared to concentrations during the first 12 h after foaling. In contrast, milk ARA concentrations were greater ( $P < 0.05$ ) in milk of mares of both the D and ED groups compared to the CORN group from 12 h to 7 days postpartum, whereas only mares of the D group continued to have greater ( $P < 0.05$ ) milk ARA concentrations at 14 and 21 days postpartum compared to mares of the CORN group (Table 4). The  $P$ -values for effects of time, treatment, and time by treatment interaction on milk LA, ALA, and ARA are reported in Table 4. Milk EPA was greater ( $P < 0.05$ ) in the mares of the ED compared to CORN group at parturition, and greater ( $P < 0.01$ ) compared to both mares in the CORN and D groups between 12 h and 21 days postpartum (Fig. 3). Milk EPA peaked ( $P < 0.05$ ) at day 7 postpartum in mares of all three groups. There were effects ( $P < 0.01$ ) of time, treatment, and the time by treatment interaction on milk EPA concentrations. Compared to the mares in the CORN group, milk DHA was greater ( $P < 0.05$ ) in mares of the D and ED groups at 12 h and 7 days postpartum with concentrations remaining greater in mares of the D group at 14 and 21 days (Fig. 3). The  $P$ -values for effect of time, treatment, and the time by treatment interaction on milk DHA were 0.04,  $< 0.01$ , and 0.55, respectively. There were no effects of treatments on total milk FA (mg/mL, data not shown).

### 3.3. IgG analysis

The concentration of milk IgG(T) was similar ( $P > 0.05$ ) among treatment groups at parturition (Table 5). At 12 h postpartum, IgG(T) concentrations were greater ( $P < 0.05$ ) in milk of mares in the D compared to CORN groups, with intermediate values for mares of the ED group (Table 5). Although there were no significant differences among treatment groups for foal plasma IgG(T) at any of the time points measured, foals of the ED group tended to have greater ( $P = 0.08$ ) IgG(T) concentrations at 12 h postpartum compared to those of the CORN group (Table 5). As expected, there was a time effect as IgG(T) concentrations were greater in plasma of foals of all treatment groups at 12 h ( $P < 0.01$ ) and 28 days ( $P < 0.01$ ) compared with plasma concentrations at birth.

### 3.4. Parturition and ovarian function

Gestation length and the total number of days of inclusion in the experiment were similar among treatment groups (Table 5). All mares had an expelling of the placenta within 3 h postpartum, and parturition occurred within a normal time frame and without



**Fig. 1.** Mare plasma (A) eicosapentaenoic acid (EPA) and (B) docosahexaenoic acid (DHA) concentrations (LS means  $\pm$  SEM) following dietary supplementation during late gestation and early lactation with corn oil (CORN), DHA (D), or EPA/DHA (ED); Sample times represent the start of supplementation 60 days prior to the expected foaling date (start), wk 2 and 6 from the start of supplementation, at parturition (part.), day 21 postpartum, and when a 35-mm follicle was first measured during the second postpartum estrous period (end); Sample point differs from other treatments (\* $P < 0.05$ ; \*\* $P < 0.01$ ); Means for D mares from start to d 21 based on  $n = 7$ , and end means based on  $n = 6$ .

**Table 3**

Foal plasma linoleic acid (LA), alpha-linolenic acid (ALA), and arachidonic acid (ARA) concentrations at birth, 7, 14, and 21 days of age when there was dietary supplementation of suckling mares with corn oil (CORN), a docosahexaenoic acid supplement (D), or an eicosapentaenoic and docosahexaenoic acid supplement (ED) during late gestation and early lactation.

Fatty acid, $\mu\text{g} / \text{mL}$	Treatment <sup>1</sup>			Pooled SEM	P-value		
	CORN	D	ED		Trt	Time	T x T <sup>2</sup>
<b>LA</b>					0.13	< 0.01	0.69
Birth <sup>3</sup>	251.17	227.57	207.57	82.43			
Day 7	1231.82	1151.76	1002.62	82.43			
Day 14	1250.18	1153.86	1007.43	85.86			
Day 21	1042.88	983.85	1007.43	85.86			
<b>ALA</b>					0.15	< 0.01	0.05
Birth	6.78	6.41	11.26	14.83			
Day 7	120.22	62.95	63.06	14.83			
Day 14	88.25	56.70	91.63	15.50			
Day 21	83.10	45.46	38.65	15.15			
<b>ARA</b>					< 0.01	< 0.01	< 0.01
Birth	84.16 <sup>a</sup>	67.56 <sup>b</sup>	56.75 <sup>b</sup>	5.84			
Day 7	42.03 <sup>a</sup>	71.79 <sup>b</sup>	50.57 <sup>a</sup>	5.84			
Day 14	35.58 <sup>a</sup>	58.66 <sup>b</sup>	39.58 <sup>a</sup>	6.15			
Day 21	30.27 <sup>a</sup>	52.44 <sup>b</sup>	30.67 <sup>a</sup>	5.84			

<sup>a,b</sup>Within a row, means lacking a common superscript differ ( $P < 0.05$ ).

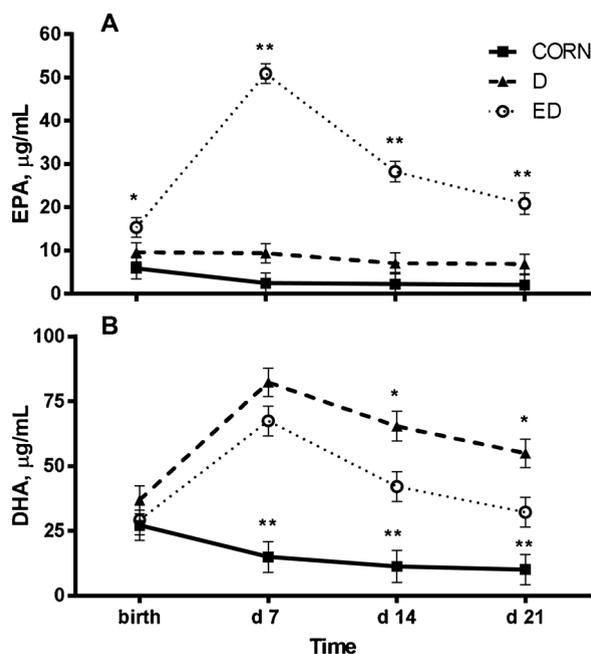
<sup>1</sup> LS means.

<sup>2</sup> Treatment x Time interaction.

<sup>3</sup> Foal plasma at birth based on  $n = 5$  for CORN,  $n = 6$  for ED, and  $n = 7$  for D; All other samples ( $n = 6$ , CORN;  $n = 7$ , D and ED diets).

complications with the exception of one mare in the CORN group with a history of mild dystocia. With the exception of one mare in the D group, all had spontaneous ovulations after parturition. Because ovulation had to be induced artificially in this one mare of the D group after day 70 postpartum, the data from this mare were not considered with statistical evaluation of the follicular data.

The number of days from parturition to the first postpartum ovulation was greater ( $P < 0.01$ ) in mares of the ED ( $22.6 \pm 2.6$  days) compared to CORN ( $12.5 \pm 2.3$  days) and D ( $13.3 \pm 2.3$  days) groups (Table 5). Likewise, there was a longer ( $P < 0.01$ )



**Fig. 2.** Foal plasma (A) eicosapentaenoic acid (EPA) and (B) docosahexaenoic acid (DHA) concentrations (LS means  $\pm$  SEM) at birth, 7, 14, and 21 days of age when diets of suckling mares were supplemented with corn oil (CORN), DHA (D), or EPA / DHA (ED) during late gestation and early lactation; \*\*Sample point differs from other treatments ( $P < 0.01$ ); \*Sample point differs from other treatments ( $P < 0.05$ ); A) \* ED greater than CORN ( $P < 0.05$ ); Means at birth based on  $n = 5$  for CORN,  $n = 6$  for ED, and  $n = 7$  for D foals; All other time points based on  $n = 6$  for CORN, and  $n = 7$  for ED and D animals.

**Table 4**

Milk linoleic acid (LA), alpha-linolenic acid (ALA), and arachidonic acid (ARA) concentrations at parturition, 12 h postpartum, and days 7, 14, and 21 of lactation for mares with dietary supplementations of corn oil (CORN), a docosahexaenoic acid supplement (D), or an eicosapentaenoic and docosahexaenoic acid supplement (ED) during late gestation and early lactation.

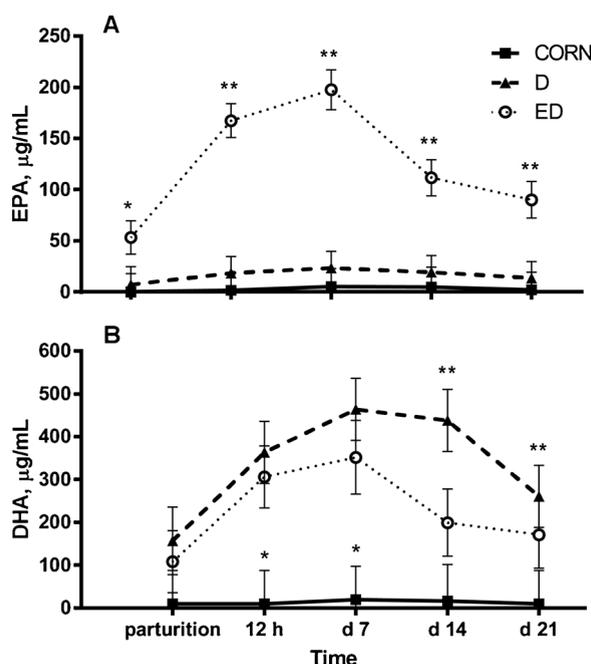
Fatty acid, µg / mL	Treatment <sup>1</sup>			Pooled SEM	P-value		
	CORN	D	ED		Trt	Time	T x T <sup>2</sup>
<b>LA</b>					0.23	< 0.01	0.72
Parturition	2510.19	2048.92	2296.03	557.33			
12 h	4083.08	3558.57	4591.33	542.77			
Day 7	4484.90	3202.70	3110.02	576.00			
Day 14	3647.55	2608.44	2306.74	543.18			
Day 21	2314.39	2237.73	1936.22	543.18			
<b>ALA</b>					0.29	< 0.01	0.11
Parturition	666.31	773.61	775.36	272.04			
12 h	1073.59	1238.36	1368.97	265.59			
Day 7	2221.87	1654.74	1722.50	280.42			
Day 14	2388.15	1130.42	1981.10	280.46			
Day 21	1940.65	1621.96	1428.49	265.25			
<b>ARA</b>					< 0.01	< 0.01	0.74
Parturition	10.21	18.88	15.72	4.69			
12 h	15.74 <sup>a</sup>	36.04 <sup>b</sup>	34.89 <sup>b</sup>	4.84			
Day 7	26.39 <sup>a</sup>	48.47 <sup>b</sup>	40.95 <sup>b</sup>	4.84			
Day 14	20.07 <sup>a</sup>	35.57 <sup>b</sup>	26.51 <sup>a,b</sup>	4.84			
Day 21	14.88 <sup>a</sup>	29.34 <sup>b</sup>	21.38 <sup>a,b</sup>	4.69			

<sup>a,b</sup>Within a row, means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> LS means.

<sup>2</sup> Treatment x Time interaction.

period for mares of the ED group before there was development of a 35 mm follicle after parturition ( $9.6 \pm 0.7$  days) than for mares of the CORN ( $6.2 \pm 0.8$  days) group. In addition, the duration of time mares in the ED group had retention of a follicle  $\geq 35$  mm prior to ovulation was greater ( $P < 0.05$ ), being  $12.7 \pm 1.8$  days compared to  $6.3 \pm 2.0$  days and  $6.0 \pm 2.0$  days for mares in the CORN and D groups, respectively (Table 5). Even though there was a longer period of follicular retention, n-3 supplementation did not affect



**Fig. 3.** Milk concentrations of (A) eicosapentaenoic acid (EPA) and (B) docosahexaenoic acid (DHA) at parturition, 12 h postpartum, and day 7, 14, and 21 of lactation in mares with dietary supplementation of corn oil (CORN), DHA (D), or EPA/DHA (ED) during late gestation and early lactation; Values are listed as LS means  $\pm$  SEM. A) \*ED greater than CORN ( $P < 0.05$ ); \*\*Sample point differs from other treatments ( $P < 0.01$ ); B) \*Sample point differs from other treatments ( $P < 0.05$ ); \*\*D greater than CORN ( $P < 0.01$ ).

**Table 5**

Mare milk and foal plasma immunoglobulin (Ig) G(T) concentrations and mare values for reproductive variables following dietary supplementation with corn oil (CORN), docosahexaenoic acid (D), or eicosapentaenoic and docosahexaenoic acid (ED) during the late gestation and early postpartum periods.

Item	Treatment <sup>1</sup>			Pooled SEM
	CORN	D	ED	
<b>IgG(T), mg/dL<sup>2</sup></b>				
Milk <sup>3</sup> at parturition	2066.8	2017.3	2408.9	336.53
Milk at 12 h postpartum	139.7 <sup>c</sup>	415.1 <sup>d</sup>	195.5 <sup>c,d</sup>	90.97
Foal <sup>4</sup> plasma at birth	16.9	11.1	7.5	9.17
Foal plasma at 12 h	240.9	296.1	318.0	29.3
Foal plasma at 28 d	169.9	161.8	158.3	19.87
<b>Reproductive variables<sup>2</sup></b>				
Gestation length, d	346.3	348.3	342.9	2.97
Max. follicle size prior to ovulation, mm	49.5	49.8	51.4	2.07
Days from parturition to a 35 mm follicle	6.2 <sup>a</sup>	7.3 <sup>a,b</sup>	9.6 <sup>b</sup>	0.77
Days with $\geq$ 35 mm follicle prior to ovulation	6.3 <sup>c</sup>	6.0 <sup>c</sup>	12.7 <sup>d</sup>	1.97
Days to 1 <sup>st</sup> post-partum ovulation	12.5 <sup>a</sup>	13.3 <sup>a</sup>	22.6 <sup>b</sup>	2.23
Max. follicle size on d 15 post-ov, mm	43.2 <sup>c</sup>	34.6 <sup>d</sup>	32.9 <sup>d</sup>	2.35
Days on trial	95.2	101.3	103.4	3.23

<sup>a,b</sup>Within a row, means lacking a common superscript differ ( $P < 0.01$ ).

<sup>c,d</sup>Within a row, means lacking a common superscript differ ( $P < 0.05$ ).

<sup>1</sup> LS means.

<sup>2</sup> Foal plasma at birth ( $n = 5$ , CORN;  $n = 6$ , ED;  $n = 7$ , D); All other IgG values ( $n = 6$ , CORN;  $n = 7$ , ED;  $n = 7$ , D); Means for all follicle variables ( $n = 6$ , CORN;  $n = 6$ , D;  $n = 7$ , ED).

<sup>3</sup> Milk IgG(T)  $P$ -values for treatment, time, and the trt  $\times$  time interaction are 0.74,  $< 0.01$ , and 0.46 respectively.

<sup>4</sup> Foal IgG(T)  $p$ -values for treatment, time, and the trt  $\times$  time interaction are 0.58,  $< 0.01$ , and 0.39, respectively.

the maximum diameter of the dominant follicle prior to ovulation (Table 5). On day 15 post-ovulation prior to the administration of OXY, the largest follicle was smaller ( $P < 0.05$ ) in mares of the ED ( $32.8 \pm 2.3$  mm) and D ( $34.6 \pm 2.3$  mm) groups compared to the CORN ( $43.2 \pm 2.5$  mm) group (Table 5).

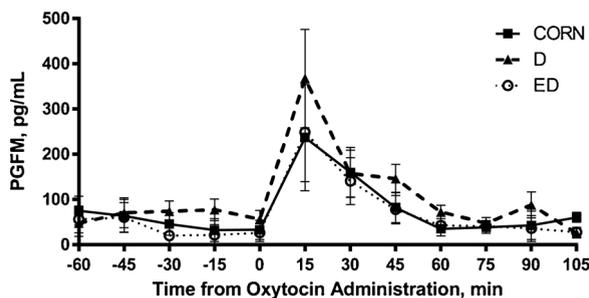


Fig. 4. Mean serum prostaglandin  $F_{2\alpha}$  metabolite (PGFM) concentrations in response to an administration of 20 IU of oxytocin (Time 0) on day 15 following the first postpartum ovulation in mares with dietary supplementation of corn oil (CORN,  $n = 6$ ), docosahexaenoic acid supplement (D,  $n = 6$ ), or eicosapentaenoic and docosahexaenoic acid supplement (ED,  $n = 7$ ); Treatments did not differ ( $P > 0.05$ ) at any time point.

### 3.5. Prostaglandin response to OXY administration

On day 15 following the first postpartum ovulation, there were no differences ( $P > 0.05$ ) among groups for PGFM concentrations during the 165 min sampling period, including the peak PGFM concentration following the OXY administration, or the time from the peak response back to baseline values (Fig. 4). The PGFM concentrations, however, were greater ( $P < 0.05$ ) immediately following the OXY administration compared to all other time points. The overall  $P$ -values for the effect of time, treatment, or the time by treatment interaction were 0.17, 0.59, and 0.68, respectively.

## 4. Discussion

Similar to results in other species, results of recent studies indicate dietary long-chain FA (LCFA) affect the FA composition of plasma in horses. Consistent with these previous findings, results of the present study indicate the dietary supplementation with marine-derived DHA or combined EPA/DHA supplementation of mare diets during late gestation and early lactation resulted in greater plasma EPA and DHA concentrations, as compared with mares fed diets supplemented with additional LA in the form of corn oil. The conversion of ALA to EPA and then DHA by elongation and desaturation enzymes has not been fully characterized in horses. In humans, this process appears to be very inefficient with less than 3% of ALA being converted to EPA (Gerster, 1998; Larsson et al., 2004). The lack of a response to feeding flaxseed oil, which contains large amounts of ALA, in increasing concentrations of DHA in horses (Hansen et al., 2002) might indicate that there is a lack of EPA to DHA conversion in horses. Results from previous and the current study, therefore, indicate that supplementing diets of horses with FA from a marine source rather than a plant source is a more effective approach to increasing the plasma concentration of LCFA.

Increased plasma ARA (Hall et al., 2004) and total n-6 FA (Woodward et al., 2005) have been previously reported in horses fed a fish oil supplement compared to dietary supplementation with corn oil. Across treatments, LA, ARA, EPA, and DHA concentrations tended to increase from the start of the present experiment until parturition, with concentrations being slightly less during the postpartum compared with prepartum period. Considering the concurrent postpartum increase seen in the milk LCFA concentrations, these results may indicate a mobilization of FA that are transferred into milk.

Results of previous research indicate that in addition to the effect on mare plasma profiles, there is an effect of EPA and DHA on mare milk composition as a result of inclusion of marine-based LCFA in the diet (Kruglik et al., 2005). Stelzleni et al. (2006) reported similar findings in mares fed encapsulated fish oil beginning at 28 days prepartum. Dietary n-3 supplementation from a marine-based source also increased the milk concentrations of EPA and DHA in sows (Fritsche et al., 1993; Meers et al., 2006), dairy cows (Petit et al., 2002; Mattos et al., 2004), and women (Olafsdottir et al., 2006). Previous evidence for this transfer to milk is consistent with finding in the current study in which the mares fed supplemented diets had greater milk concentrations of EPA and DHA, or DHA alone, compared to the mares fed the corn oil supplemented diet. Considered together, the data clearly indicate that milk EPA and DHA content can be affected by supplementation both before and after parturition. These findings indicate there is an opportunity to investigate potential effects of LCFA on foal growth and development.

Of interest, relative to the primary hypothesis for the present experiment, was whether maternal DHA or EPA/DHA supplementation during gestation could alter the foal FA status, at least as reflected in foal plasma. In plasma samples obtained prior to the foal suckling for the first time, EPA was greater in foals of ED than CORN group, providing strong evidence for an *in utero* transfer of EPA into the foal plasma. Compared to controls, Kruglik et al. (2005) also reported there was an increased plasma EPA and DHA at birth, and through day 49 postpartum, in foals from mares fed a similar EPA/DHA supplement as that of the present study during late pregnancy. In the present study, foal plasma EPA and DHA concentrations remained greater than those of foals from mares in the CORN group through the first 21 days postpartum. Stelzleni et al. (2006) also reported there were greater plasma EPA and DHA concentrations in foals suckling mares fed encapsulated fish oil beginning 28 days pre-partum.

Supplementation with n-3 FA has immuno-modulating effects in a number of species, potentially affecting the humoral immune system (Wu and Meydani, 1998; Harbig, 2003; Blümer and Renz, 2007). Jackson et al. (1995) reported that IgG concentrations in colostrum were less in sows supplemented with corn oil compared to control diets, perhaps indicating reduced antibody transfer. In addition, chicks fed diets containing fish oil had greater serum IgG concentrations than chicks supplemented with sunflower, animal,

or linseed oils (Wang et al., 2000). In horses, results of the effects of n-3 supplementation on antibody transfer into colostrum are inconsistent. There are greater IgG(T) concentrations at parturition in mares fed a marine-based EPA and DHA supplement starting 60 days prior to the expected foaling date (Kruglik et al., 2005). Stelzleni et al. (2006), however, reported there was no effect on IgG concentrations in colostrum from mares fed encapsulated fish oil that provided 6 g total n-3 FA/100 kg BW with supplementation beginning at 28 days prepartum. The current findings are consistent with those in a previous study. There was no difference in IgG(T) concentrations of colostrum among treatment groups at parturition in the present study. There, however, was a tendency for mares of the D group to have greater milk IgG(T) concentrations at 12 h postpartum compared to mares of the CORN and ED groups. There was no difference between foal plasma IgG(T) concentrations at any time among treatment groups in the present study or in a previous study (Kruglik et al., 2005). The inconsistency in colostrum results among studies may be due to several factors, including differences in the duration of n-3 supplementation, amount of incorporation in the diet, or differences between a plant and marine n-3 source. Individual variation and sample size also must be taken into account as possible sources of inconsistencies in results.

In the current study, all mares had progressive follicular growth characteristics in the early postpartum period. There was no difference between mares of the CORN and D groups in the length of time required for a follicle to develop to be 35-mm in diameter after parturition. The mares of the ED group had a longer interval from parturition to the development of a 35-mm follicle compared with mares fed corn oil, indicating perhaps a slower follicular growth rate in mares supplemented with EPA and DHA. Similarly, mares of the ED group had a longer period before ovulation occurred following parturition (mean of 22.6 days) and there was retention of a dominant follicle for a longer period prior to ovulation than in mares of both CORN and D groups. After assessment of 470 mares during the first postpartum estrous period, Loy (1980) reported that 93% of mares had ovulations by day 15 postpartum and 97% had ovulations by day 20. Although the number of mares per treatment group was small, to our knowledge, the present study is the first where there was assessment of a possible effect of EPA and DHA supplementation on follicular dynamics in mares. In rats fed a sardine oil diet high in n-3 PUFAS, ovulation rate was greater compared to controls and rats fed a high n-6 diet (Trujillo and Broughton, 1995). It was speculated that there was a greater ovulation rate in rats fed a diet with n-3 due to a greater production of PGE<sub>3</sub> or a lesser PGE<sub>2</sub> synthesis, with the suppression of ovulation in the rats fed n-6 possibly occurring because of the greater production of PGE<sub>2</sub>.

The mechanism for the follicular retention in mares of the ED group in the present study is unclear, but may be related to a decrease in ARA substrate availability, and subsequent decrease in PG synthesis, as a result of increased dietary EPA (Santos et al., 2008). The production of PGE<sub>2</sub> and PGF<sub>2</sub>α by the pre-ovulatory follicle is essential for mammalian ovulation (Murdoch et al., 1986; Bridges and Fortune, 2007; Stouffer et al., 2007). These two eicosanoids are produced from the substrate ARA after the conversion of LA to ARA by a desaturation and elongation process. It has been suggested that increasing dietary concentrations of n-3 PUFAs, such as ALA or EPA, may inhibit the incorporation of ARA into membrane phospholipids, thereby suppressing the synthesis of ARA-derived series 2 prostanoids, such as PGE<sub>2</sub> and PGF<sub>2</sub>α, and enhancing the synthesis of EPA-derived series 3 prostanoids (Mattos et al., 2002; Wathes et al., 2007).

In dairy cows, the effect of n-3 supplementation on PG synthesis is inconsistent. Plasma PGFM concentrations were reduced in fish oil-fed cows during the first few days postpartum (Mattos et al., 2004), or on day 15 of the estrous cycle (Mattos et al., 2002). In contrast, there was no effect of fish oil (Wamsley et al., 2005; Heravi Moussavi et al., 2007) or a diet high in ALA (Robinson et al., 2002) on plasma PGFM concentrations in dairy cows at the end of the luteal phase. In the current study, mares were catheterized on day 15 following the first postpartum ovulation, and plasma PGFM concentrations were quantified in response to an OXY administration. In all groups, there was an expected increase in PGFM concentrations following the administration, however there was no difference in the PGFM response among groups. Even though changes in peripheral concentrations of PGFM were not detected following supplementation, these results do not lead to a discounting of the possibility of an alteration in PG synthesis or metabolism by the ovary.

A second, and perhaps more plausible, explanation for the increased follicular lifespan in the mares of the ED group may be due to changes in the concentration of insulin-like growth factor 1 (IGF-1). Although not measured in the current study, results from subsequent research in the laboratory where the present research was conducted indicate that EPA and DHA supplementation of diets to non-pregnant mares during the breeding season reduced ( $P < 0.05$ ) serum and follicular fluid concentrations of IGF-1 (Buist et al., 2014). The dietary supplementation resulted in a similar amount of DHA (10.08 g/d) and a greater amount of EPA (18.45 g/d), but there was feeding for a shorter time period compared to what occurred in the current study. Concentration of IGF-1 is positively correlated with follicle selection and maturation, and is thought to be an important factor in the ovulatory process in horses (Ginther et al., 2001, 2008). The initial follicular retention, coupled with the smaller follicles measured on day 15 post-ovulation in both supplemented groups compared to mares fed the corn oil supplemented diet, supports a potential effect of dietary EPA and DHA on follicle maturation. Results from these two studies may indicate that there is an intriguing difference in response when EPA is fed alone, or EPA is fed in combination with DHA, on the capacity of these diets to modify reproductive processes in mares.

## 5. Conclusions

Dietary supplementation of mares with marine-derived DHA or EPA/DHA during late gestation alters the FA composition of the plasma, resulting in a greater concentration of EPA and DHA incorporation into the milk compared to mares with dietary supplementation with LA-rich corn oil. Foals that are in utero and that are suckling their dams had a transfer of LCFA into their blood. At present, the beneficial effects for foals, if any, remain to be determined. Dietary supplementation of EPA alone, or in combination with DHA, also may affect ovarian follicular growth and the timing of ovulation during the first postpartum estrous cycle.

## Conflict of interest

None.

## Acknowledgements

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