Effect of linseed feeding on blood metabolites, incidence of cystic follicles, and productive and reproductive performance in fresh Holstein dairy cows

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ABSTRACT

This study was done to investigate the effect of feeding linseed on blood metabolites, incidence of cystic follicles, resumption of postpartum ovarian cyclicity, pregnancy rate, milk production, and composition in fresh Holstein dairy cows. A total of 399 dairy cows were assigned randomly to 2 diets. Diets contained either protected palm oil (CON) or extruded linseed (LIN) and were fed from calving to d 40 postpartum. Ovaries of each cow were examined on d 10, 20, 30, and 40 after parturition (parturition = d 0) by transrectal ultrasonography to determine follicular development, ovarian disorders, and cyclicity. Blood samples were collected at 14-d intervals for 6 wk starting on the day of parturition to determine plasma concentrations of glucose, β -hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), and blood urea N (BUN). Results showed plasma glucose concentration was affected by the diets and was greater in the LIN treatment, but BHBA, NEFA, and BUN concentrations were similar among treatments. Dietary treatments had no significant effect on milk production and composition except milk fat percentage that significantly decreased in cows fed LIN (3.55%) compared with those fed with CON (4.17%). Plasma progesterone concentrations were greater in LIN treatment than CON treatment (1.31 ± 0.09 vs. 0.87 ± 0.09) at early postpartum. The resumption of cyclicity and onset of estrus were influenced by treatments and reduced by 7 d in LIN treatment compared with CON treatment. Cows fed diets enriched in LIN fatty acids had a lesser incidence of cystic follicles. Treatments did not differ significantly in terms of the number of days open, number of services per pregnancy, and pregnancy rate. In conclusion, feeding linseed immediately after parturition decreased milk fat and incidence of cystic follicles, increased progesterone concentrations early postpartum, and caused earlier resumption of cyclicity but did not affect pregnancy rate.

Key words: fertility, Holstein dairy cow, linseed, postpartum, reproduction

INTRODUCTION

Negative energy balance in dairy cows after calving is characterized by low blood concentrations of glucose, insulin, and IGF-1 and high blood concentrations of NEFA and BHBA (Ingvartsen and Andersen, 2000). These shifts in blood metabolites and hormones might compromise ovarian function and fertility. Feeding diets that promote increases in plasma glucose and insulin may improve the metabolic and endocrine status of cows in early lactation (Santos et al., 2004).

In recent years, dietary fats rich in n-3 PUFA have been widely used to enhance reproductive performance in dairy cows (Silvestre et al., 2011; Caldari-Torres et al., 2011; Dirandeh et al., 2013a; Badiei et al., 2014) by improving dietary energy density (Ferguson et al., 1990); influencing follicular growth and ovulation (Silvestre et al., 2011; Dirandeh et al., 2013b); increasing the number, diameter, and the life span of the corpus luteum (CL; Garcia-Bojalil et al., 1998); increasing concentrations of progesterone (Santos et al., 2008; Dirandeh et al., 2013b); early postpartum ovarian cyclicity (de Veth et al., 2009); prevention of luteolytic signals around maternal recognition of pregnancy (Mattos et al., 2000; Dirandeh et al., 2013a); and improved embryo quality (Cerri et al., 2004).

Linseed and its oil have been used widely as a conventional source of n-3 PUFA that contain >500 g of α linolenic acid (ALA) per kilogram of total fatty acid (Moallem, 2009). Linseed is incorporated into dairy feeds to improve reproduction and milk quality in dairy cows (Dirandeh et al., 2013b). Feeding protected linseed or its protected oil may enhance reproductive performance and increase the n-3 PUFA content of milk fat (Gonthier et al. 2005). Recent studies have shown a 16% increase in pregnancy rate in cows fed linseed compared with those fed palm oil (Dirandeh et al., 2013a).

Because ovarian activity usually returns within the first 4 wk of calving, initiating fat feeding prepartum would allow the absorbed fatty acids to influence early

Received August 26, 2014.

Accepted November 10, 2014.

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ovarian activity (Santos et al., 2010). The incidence of cystic follicles is higher in high-producing dairy cows and is associated with lower percentages of pregnancy per AI and increases interval to pregnancy, culling, and economic losses. Cows with cystic follicles take 6 to 11 d longer to first service and 20 to 30 more days to conception (Fourichon et al., 2000).

Although many experiments have determined the effects of n-3 PUFA supplementation on productive and reproductive performances, little information is available on the effects of feeding linseed immediately postpartum on parameters influencing reproduction such as the incidence of cystic follicles and resumption of postpartum ovarian cyclicity. Therefore, the objectives of the current study were to determine the effect of linseed on blood metabolites, plasma concentrations of progesterone, resumption of postpartum ovarian cyclicity, incidence of cystic follicles, milk production, and composition and pregnancy rate in fresh Holstein dairy cows.

MATERIALS AND METHODS

Cows and Treatments

The experiment was conducted on a commercial dairy farm in Iran from November 2011 to April 2012 using 399 Holstein dairy cows (yielding 30.4 ± 0.3 kg of milk/d, 199 primiparous and 200 multiparous) with no overt clinical illnesses. The experiment was conducted from calving until wk 6 of lactation. Cows were blocked according to calving date, parity, and BCS and randomly assigned to 1 of 2 isonitrogenous and isoenergetic total mixed diets (Table 1). No difference was present among groups (mean $\pm$ SEM) in parity (2.5 ± 1.4) or BCS at calving (3.2 ± 0.07). All cows calved within 3 wk. Treatments included (1) protected palm oil (**CON**; Magnapac, Norel S.A., Suez, Egypt) and (2) extruded linseed (**LIN**, OmegaLin; Nutri Advance, Ploufragan, France). Diets were fed twice daily (0700 and 1700 h) for ad libitum intake (10% of refusals on an as-fed basis) during the whole experiment. Cows were milked 3 times daily at 0600, 1400, and 2200 h. All cows had a similar dry period condition and were fed a diet primarily consisted of alfalfa, corn silage, barley grain, and wheat bran in far-off. The composition of close-up diet has been shown in Table 1. Mature cows and heifers were housed together during close-up and postfresh periods. Twenty-three days before the expected calving date, mature cows and heifers were moved to the close-up group in a twice per week schedule.

Analysis of Feed

Total mixed ration were sampled each week and pooled each month and analyzed. Briefly, the DM

of the feed sample was determined by placing it in a drying oven at 100°C for 48 h (AOAC, 1990; method 930.15). Crude protein was determined using the Dumas method and a Leco FP-528 (Leco Corporation, St. Joseph, MI). Acid detergent fiber and NDF concentrations were determined (Van Soest et al., 1991).

Milk Sampling and Analysis

Milk production was recorded at every milking. Milk samples were taken weekly (Sunday and Tuesday; 3 times per day, milk samples pooled on a yield basis so only 1 sample was taken per week) during experiment and analyzed for fat, protein, and lactose by infrared spectroscopy (AOAC International, 2000; method 972.16). The Milkoscan 4000 uses infrared technology to analyze the components of milk.

Blood Sampling and Processing

Blood samples (only for 30 cows within each treatment, a total of 60 cows) were collected at 14-d intervals for 6 wk starting 24 h after parturition (d = 0) for determining glucose, BHBA, NEFA, and BUN. Blood samples (only for 20 cows per treatment, a total of 40 cows) were collected daily from d 10 to 25 postpartum for determination of plasma progesterone concentrations. Blood samples (10 mL) were collected, by coccygeal venipuncture into anticoagulated (EDTA) evacuated tubes (Monoject, Sherwood Medical, St. Louis, MO), before the feeding and were kept on ice. All blood samples were centrifuged for 10 min at $2,000 \times g$ at 4°C, and plasma was harvested and transferred into 2-mL microcentrifuge tubes and kept at -20°C until assayed. Plasma concentrations of glucose (Glucotrend, Roche, UK), NEFA (FA 115, Randox Laboratories Ltd., Antrim, UK), and BHBA (Abbott Diabetes Care Ltd., Witney, UK) were determined enzymatically using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). Intra- and interassay coefficients of variation were $<5\%$. Plasma progesterone concentrations were analyzed by ELISA following the manufacturer's instructions (DRG, Frauenbergstr, Marburg, Germany).

Ultrasonographic Examination and Estrus Detection

Cows were synchronized with 2 PGF_{2 α} injections 14 d apart started at d 45 postpartum. The voluntary waiting period of the dairy herd was 60 d, and cows were inseminated based on a voluntary waiting period. Pregnancy diagnosis was performed by ultrasonography at d 32 after AI. Cows diagnosed pregnant at d 32 were reexamined at d 60 ± 5 after AI to confirm pregnancy (Dirandeh et al., 2014).

Table 1. Ingredient and chemical composition of experimental diets and diet of postexperiment period

Item	Close-up	CON	LIN	After 40 DIM
Ingredient composition, % of DM				
Alfalfa hay	20.4	22.5	22.5	22.2
Corn silage	37.6	16.2	16.2	18.5
Beet pulp		6.3	6.3	0
Ground barley grain	12.7	11.2	11.2	17.4
Ground corn grain	9.0	14.0	14.0	13.4
Wheat bran	5.4	0	0	0
Cottonseed whole	2.1	8.4	8.4	8.8
Canola meal	6.0	0	0	0
Soybean meal 44% CP	3.3	12.1	9.2	12.2
Corn gluten meal	0.5	2.5	2.5	2.0
Linseed (OmegaLin) ¹	0	0	4.5	0
Magnapac ²	0	1.6	0	1.7
Mineral-vitamin mix ³	0.8	0.6	0.6	0.4
Anionic salt	1.1	0	0	0
Dicalcium phosphate	0	0.2	0.2	0.2
Salt	0	0.2	0.2	0.2
Sodium bicarbonate	0	1.1	1.1	2.0
Calcium carbonate	0	0.5	0.5	0.2
Bentonite	0	0.7	0.7	0.8
Glycolaine ⁴	1.1	1.9	1.9	0
Chemical composition (DM basis)				
NE _L , ⁵ Mcal/kg	1.50	1.68	1.67	1.66
CP, %	13.4	16.8	16.4	16.8
NDF, %	40.0	30.3	31.1	30.7
Forage NDF, %	31.1	18.4	18.6	19.6
ADF, %	25.3	20.1	20.5	20.2
NFC, ⁴ %	37.9	38.7	38.5	40.9
Ether extract, %	3.0	5.0	5.1	5.25
RDP, ⁴ %	10	11.5	11.0	11.7
RUP, ⁴ %	3.4	5.3	5.4	5.1

¹Omegalín consisted of 100 g/kg of DM wheat meal and 900 g/kg of DM extruded Tradi-Lin grain (Valorex, Combourtille, France). Tradi-Lin uses a patented process characterized by a preliminary maturing step of specific duration (10–30 min) and temperature (<100°C), level of steam incorporation, and the mounting of the extruders (patent no. EP 1 021 960 B1).

²Calcium salts of fatty acids distillate from palm oil (Magnapac, NOREL S.A., Suez, Egypt). Analysis: 84%. Fatty acid composition (NOREL S.A., g/kg of total fatty acids): C14:0, 1.5%; C16:0, 44%; C18:0, 5%; C18:1, 40%; C18:2, 9.5%.

³Provided (per kg of DM): 500,000 IU of vitamin A, 100,000 IU of vitamin D, 1,000 mg of vitamin E, 9,000 mg of P, 195,000 mg of Ca, 2,000 mg of Mn, 55,000 mg of Na, 2,000 mg of Zn, 2,000 mg of Fe, 280 mg of Cu, 100 mg of Co, 100 mg of Br, 1 mg of Se, and 3,000 mg of antioxidant.

⁴DSL Chemical (Shanghai) Co. Ltd., Shanghai, China.

⁵Calculated using the NRC (2001) values.

Ovarian follicles were monitored from d 10 postpartum until detecting the first postpartum ovulation (30 ± 3.8) every other day to determine diameter of ovulatory follicle by transrectal ultrasonography using a real-time linear scanning ultrasound diagnostic system fitted to a Falco 100 B-mode scanner (ECM AL, Agrosan, Angoulême, France) as previously described by Dirandeh et al. (2009). The horizontal and vertical diameters of follicle were recorded and the average was used in the analysis; to determine ovarian disorders and cyclicity, ovaries of each cow were examined on d 10, 20, 30, and 40 after parturition (parturition = d 0).

The cows were observed visually around the estrus period for estrus detection twice daily at approximately 12-h intervals for a minimum of 30 min per period. Ovulation was confirmed by the absence of

a large (diameter ≥ 10 mm) follicle that had been detected at the previous examination, and subsequent CL formation (Dirandeh, 2014). Anovulatory follicles ≥ 25 mm in diameter that persisted for at least 10 d in the absence of a CL were defined as cysts (Garverick, 1997).

Statistical Analysis

Data were tested for normal distribution of the residuals by the PROC UNIVARIATE procedure of SAS (v.8.0, SAS Institute, Cary, NC). Data on milk yield and composition, plasma concentrations of progesterone, and metabolites were analyzed as repeated measurements using PROC MIXED of SAS with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \tau_k + (\alpha\beta)_{ij} + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + e_{ijk},$$

where μ is the population mean; α_i is the treatment effect; β_j is the fixed effect of parity; τ_k is the effect of sampling day or time; $(\alpha\beta)_{ij}$ is the interaction effect of treatment and parity; $(\alpha\tau)_{ik}$ is the interaction effect of treatment and sampling day or time; $(\beta\tau)_{jk}$ is the interaction effects of parity and sampling day or time; $(\alpha\beta\tau)_{ijk}$ is the interaction effects of treatment, parity, and sampling day or time; and e_{ijk} is the residual error. Significance was declared at $P < 0.05$.

Interval between calving and first artificial insemination and the number of days open were analyzed by survival analysis using the product limit method of the Kaplan-Meier model by the LIFETEST procedure of SAS. Significance and tendencies were declared at $P < 0.05$ and $P < 0.10$, respectively, unless otherwise indicated.

RESULTS AND DISCUSSION

Milk Production and Composition

Average DMI were 20.3 and 20.4 kg/d for the CON and LIN groups, respectively. No increase in the overall milk yield was observed during the entire period of linseed feeding ($P = 0.37$, Table 1). Milk yield increased from calving until wk 6 postpartum in both groups ($P < 0.05$, Table 1). Results on the effect of linseed supplementation on milk yield have been controversial. Some studies have reported similar milk yields for cows supplemented or not with LIN (Gonthier et al., 2005; Dirandeh et al., 2013a) and others have shown an increase in milk yield when cows were fed LIN (Petit, 2002; Zachut et al., 2010). Zachut et al. (2010) reported milk yield until 100 DIM was 6.4% greater in the cows fed extruded linseed than in the control cows.

The lower milk fat proportion for cows fed LIN compared with those fed CON that reported in present study ($P = 0.02$, Table 2) was consistent with previous studies (Chilliard et al., 2009; Zachut et al., 2010; Dirandeh et al., 2013a) and might be the result of the higher intake of PUFA by LIN-fed cows. According to Bauman and Griinari (2001), biohydrogenation of PUFA by rumen microbes leads to milk fat depression and linseed contains around 500 g/kg of total FA as linolenic acid (Fuentes et al., 2008). The first rumen biohydrogenation intermediate shown to affect milk fat synthesis is *trans*-10,*cis*-12 CLA (Baumgard et al., 2000). de Veth et al. (2004) reported a curvilinear relationship between increasing *trans*-10,*cis*-12 CLA dose and the reduction in milk fat yield. Chilliard et al. (2000) showed interest in the role of *trans* isomers of 18:1 in mediating the

milk fat depression associated with certain dairy diets. Long-chain n-3 PUFA present in linseed appear to affect rumen bacteria that catalyze the terminal step in rumen biohydrogenation and cause a *trans*-10 FA to be produced (Griinari et al., 1998). Yields of fat and fat-corrected milk were not affected by treatment ($P = 0.22$, Table 2). Lower milk fat proportion also may be the result of the linseed product that was fed, which was the extruded form. Indeed, extruded linseed fed at 14.8% of the diet decreased fat concentration from 4.11 to 3.53% in late-lactating dairy cows compared with those fed a control diet (Martin et al., 2008). Decreased milk fat percentage was also observed by Chilliard et al. (2009) in cows fed a supplement containing 70% extruded linseed at 21.2% of the diet DM. Dirandeh et al. (2013a) reported milk fat concentration declined from 3.84 to 3.50% from wk 0 to 8 in cows fed linseed. Hutchinson et al. (2012) reported that CLA decreased milk fat percentage.

Milk protein percentage and yield did not differ between the dietary groups in our study ($P = 0.47$, Table 2). No difference was found in lactose in the milk ($P = 0.64$, Table 2). The lack of a linseed effect on milk protein concentration in the present study probably was related to the low inclusion rate of linseed (45 g/kg of DM). Reports in the literature about the effects of linseed supplementation on the concentration of milk protein are inconsistent. Some studies have reported an increase in protein concentration (Petit, 2002; Fuentes et al., 2008), no effect (Petit and Benchaar, 2007), or a decrease (Ward et al., 2002). Schingoethe (1996) reviewed the effect of diet on protein concentration in the milk of dairy cows and concluded that it is easier to increase protein yield than concentration. Supplemental fat that increases milk yield will usually increase milk protein yield but reduce protein concentration (CSIRO, 2007), probably due to a dilution effect.

Blood Metabolites and Progesterone Concentrations

No significant differences were found among treatments for average plasma concentrations of metabolites ($P > 0.05$, Table 2) that were consistent with previous studies (Dirandeh et al., 2013a; Badiei et al., 2014). However, plasma glucose concentrations ($P = 0.01$) were higher in the LIN treatment compared with the CON treatment (Table 2). Dietary treatments had no effect on plasma concentrations of NEFA ($P = 0.75$) and BHBA ($P = 0.62$). No difference was observed among treatments from calving until wk 6 postpartum ($P > 0.05$, Table 1). Blood concentration of NEFA is an index of body fat mobilization (Roberts et al., 1981) and is related to the energy balance of dairy cows. Bertics et al. (1992) reported that DMI is inversely related

Table 2. Milk production and composition and blood metabolites of Holstein dairy cows fed diets based on protected palm oil (CON) or extruded linseed (LIN) from calving until wk 6 postpartum

Item	Treatment		SEM	P-value		
	CON	LIN		Treatment	Time	Treatment × time
Milk, kg/d	39.3	40.1	2.1	0.37	0.001	0.54
3.5% FCM, kg/d	42.9	40.4	2.6	0.54	0.01	0.37
Fat, %	4.17	3.55	0.20	0.02	0.001	0.17
Fat yield, kg/d	1.60	1.42	0.11	0.22	0.25	0.34
Protein, %	3.22	3.28	0.07	0.47	0.78	0.48
Protein yield, kg/d	1.26	1.30	0.06	0.19	0.23	0.67
Lactose, %	4.65	4.59	0.09	0.64	0.45	0.75
Lactose yield, kg/d	1.70	1.79	0.11	0.55	0.24	0.38
Blood urea nitrogen, mg/dL	16.8	14.4	1.32	0.10	0.36	0.06
Glucose, mg/d	51.0	57.0	1.09	0.01	0.67	0.71
BHBA, mmol/L	0.62	0.51	0.16	0.62	0.44	0.51
NEFA, mmol/L	0.36	0.38	0.03	0.75	0.74	0.32

to concentrations of NEFA and BHBA in plasma and liver.

Results showed average plasma progesterone concentrations was greater in cows fed LIN compared with those fed CON (1.31 ± 0.09 vs. 0.87 ± 0.09 ng/mL; $P = 0.01$) at early postpartum. Ruminants fed with protected PUFA were reported to show increased concentrations of progesterone during the luteal phase of the estrous cycle (Walsh et al., 2011). Dirandeh et al. (2013a,b) reported mean serum progesterone concentrations were greater during the luteal phase (d 6 to 16 of cycle, $P < 0.03$) and at the time of insemination in cows fed linseed (0.80 ± 0.05 ng/mL) than in cows fed palm oil (0.15 ± 0.05 ng/mL). Previous works has suggested that increased progesterone in plasma may not be due to the increased uptake of cholesterol and synthesis by luteal cells, but rather due to the reduction in P_4 disappearance from the plasma (Hawkins et al., 1995). Moreover, increased concentration of progesterone has been reported in cows given supplements of CLA (Castañeda-Gutiérrez et al., 2007), which was related to greater circulating IGF-I. Indeed, IGF-I plays a significant role in follicular and luteal development (Perks et al., 1999), promoting proliferation, progesterone production, and increasing LH binding sites of bovine thecal cells. As suggested for fish meal (Mattos et al., 2004), PUFA of the LIN could have reduced the sensitivity of the CL to $PGF_{2\alpha}$, or reduced uterine secretion concentration of $PGF_{2\alpha}$ that delayed the completion of functional luteolysis, resulting in a supplementary antiluteolytic effect.

Diameter of Ovulatory Follicle

The ovulatory follicle (before the first AI) was larger in cows fed LIN compared with those fed CON (15.8 ± 0.74 vs. 13.6 ± 0.66 mm; $P < 0.05$). Dairy cows fed

diets enriched in PUFA had increased dominant follicle diameters compared with the cows fed a diet enriched with MUFA (Bilby et al., 2006), suggesting that PUFA were most effective compared with MUFA. Other studies showed that the size of the dominant follicle was increased in cows that consumed a diet rich in long-chain fatty acids from linseed (Dirandeh et al., 2013a) or soybean oil (Garnsworthy et al., 2008). Cows fed with rolled linseed enriched with ALA (56.7% of total FA) had larger mean diameter of the ovulatory follicle (16.9 vs. 14.1 mm) than the cows fed with rolled sunflower seed (0.1% ALA; Ambrose et al., 2006). These studies clearly indicate the differential effects of FA profile on follicular growth. Cholesterol is the precursor of all steroids and increased substrate availability may increase follicular steroid synthesis (Carroll et al., 1990).

Resumption of Ovarian Activity and Reproductive Performances

Treatment affected time to postpartum resumption ($P = 0.02$) and was 6.5 d earlier in the LIN group compared with the CON group (Table 3). Consistent with Juchem et al. (2010) and Silvestre et al. (2011), this study indicates that lipid supplements differing in fatty acid profile influenced the resumption of cyclicity in lactating dairy cows. Prolonged luteal phase and delayed first ovulation were 2 important ovarian dysfunctions that delayed postpartum resumption of cyclicity in high-producing dairy cows (Shrestha et al., 2004). Time to postpartum resumption of ovarian activity was reduced by 7 d in Holstein cows supplemented with 500 g/d of vegetable oil (60% PUFA) compared with a control group (Marín-Aguilar et al., 2007). Time to first ovulation postpartum was reduced by 8 d when dairy cows were supplemented with 8 g/d of dietary PUFA (*trans*-10, *cis*-12 CLA; de Veth et al., 2009).

Table 3. Effect of linseed on fertility responses of Holstein dairy cows diets based on protected palm oil (CON) or extruded linseed (LIN) from calving until wk 6 postpartum

Item	CON	LIN	P-value
Cystic ovary, %	5.6	4.4	0.03
Day to first service	66.8	60.3	0.02
Open days	126.54	124.30	0.65
Conception rate at first AI, %	48.2	47.3	0.98
Proportion pregnant, %	92.6	89.0	0.38

These observations demonstrate that PUFA supplementation may influence the resumption of postpartum ovarian cyclicity. Recent studies reported no difference in the proportion of cycling cows at 65 d after calving when 699 dairy cows were fed 400 g of fatty acids from either tallow or palm and fish oil containing Ca-PUFA. Subsequently, feeding Ca-PUFA of palm oil or a blend of C18:2n-6 and *trans*-octadecenoic FA, from 25 d before to 80 d after parturition, resulted in similar mean interval to first postpartum ovulation (31.3 d; Juchem et al., 2010).

Number of days open, conception rate at first AI, and proportion of pregnant cows were not different among treatments ($P > 0.05$, Table 3). This agrees with the results of Dirandeh et al. (2013a), who reported no difference in pregnancy rate at first insemination and conception rate per AI for cows fed soybeans, linseed, or palm oil. However, percentage of pregnancy at d 120 tended ($P = 0.08$) to be higher for cows fed n-3 FA than for those fed palm oil (66.7 vs. 50.91%). Treatments did not affect interval from calving to pregnancy ($P > 0.05$). Hutchinson et al. (2012) reported CLA did not affect interval from calving to conception. Results of the present study showed mean ($\pm$ SEM) days to pregnancy for CON and LIN were, respectively, 103.8 ± 5.0 and 97.2 ± 4.8 (Figure 1). Similar to our results, Fuentes et al. (2008) reported linseed did not affect interval from calving to pregnancy (median interval from calving to pregnancy was 92 d for cows fed linseed).

Cystic Follicles

The incidence of cystic follicles was affected ($P = 0.04$) by the source of fatty acids and cows fed LIN treatment had a lower incidence of ovarian cysts that was consistent with Colazo et al. (2009). Many factors are associated with the development of ovarian follicular cyst in cattle including metritis, retained fetal membrane, and delayed uterine involution (Peter, 2004), reduced LH pulsatility associated with negative energy balance (Ribadu et al., 2000), and hypoinsulinemia (Vanholder et al., 2005). Interestingly glucose concentration was higher in cows fed LIN that could result in higher insulin concentration. We reported linseed

feeding after calving improved insulin resistance via affecting somatotrophic axis gene expressions and increase insulin concentration (unpublished data). Therefore, hypoinsulinemia may be a predisposing factor to cystic follicle development in the present study. Another mechanism may be related to the higher diameter of ovulatory follicles in LIN treatment that, due to higher estradiol production, can cause LH surge. Growth of ovarian antral follicles beyond 2 to 3 mm in diameter was largely dependent on FSH, with the final growth and maturation becoming LH dependent. As well as playing a secondary role in follicular responsiveness to FSH, LH is the trigger for ovulation itself. In response to the switch in estrogen-mediated feedback from negative to positive, the LH mid-cycle surge is created. This activates a whole cascade of inflammatory responses in the dominant follicle leading to the breakdown of the follicular boundary wall and the escape of the oocyte with its cumulus oophorus (Campbell et al., 1995). Simões et al. (2012) reported higher follicle diameters increased the ovulation rate and the gene expression of LHR isoforms (M1 + M2 + M3 + M4) in granulosa cells of follicles from Nelore cows.

In conclusion, feeding LIN during early lactation decreased the incidence of cystic follicles that could be related to increased follicle diameter, and influenced the resumption of cyclicity via increased progesterone

Figure 1. Survival curves for interval from calving to pregnancy in cows fed protected palm oil (CON) or extruded linseed (LIN). Nonpregnant cows at the end of the study were censored. Median interval from calving to pregnancy was 87 and 87 d for CON and LIN, respectively ($P = 0.90$). Mean ($\pm$ SEM) days to pregnancy for CON and LIN were, respectively, 103.8 ± 5.0 and 97.2 ± 4.8 d.

concentrations. Moreover, feeding LIN decreased yield of milk fat in the first 40 d of lactation.

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