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Effect of Supplemental Fish Meal on Reproduction and Immunology Responses in Early Lactating Holstein Dairy Cows

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Abstract: The study was designed to test the effect of supplemental Fish Meal (FM) on first 60 days cumulative milk production, interval to postpartum inseminations, number of days open, follicular dynamic and complete blood counts in early lactating Holstein cows. From 21 days before predicted calving time to 35 day in milk (DIM), cows were fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods. Diets were formulated to be isoenergetic and provide similar amounts of NEL and NFC using the Cornell Net Carbohydrate and Protein System. Ovarian follicular dynamics were monitored using a 7.5 MHz rectal transducer. The first 60 days cumulative milk production and 305 days adjusted milk yield were similar among the diets. Number of follicles, maximum diameter of 1st dominant follicle on day 10 and 14 postpartum, number of days until dominant follicle detection and number of days until detection of a follicle ≥10 mm were not different between the diets. The interval from calving to 1st, 2nd and 3rd inseminations and also number of days open were all similar among the dietary groups. Complete blood counts were similar in week preceding calving. Except than neutrophils (2374 and 4181±551, respectively) which tended to be significant ($p = 0.06$), other blood cells were not affected by diet. Results from this experiment demonstrate that dietary supplementation with fish meal from -21 to 35 day postpartum had no effect on milk yield, follicular dynamic and complete blood count.

Key words: Dairy cow, fish meal, follicular dynamic, reproduction and immunology, Hoistein dairy cows

INTRODUCTION

The transition phase, typically defined as 3 week before to 3 week after parturition, is viewed as a critical time in the lactation cycle of a dairy cow. During this period, the cow experiences a series of nutritional, physiological and social changes and is more vulnerable to infectious and metabolic diseases (Overton and Waldron, 2004; Huzzey *et al*., 2005). During early lactation Dry Matter Intake (DMI) lags behind the nutrient requirements for milk yield; consequently, lipids, proteins and minerals are mobilized from body stores to support milk synthesis (Heravi Moussavi *et al*., 2007b). So, the onset of lactation in the dairy cow is characterized by a dramatic increase in the nutrient demands for milk synthesis, which leads to negative energy balance in early lactation. Extreme negative energy balance predisposes the cow to the occurrence of several periparturient diseases and health problems that can impact milk production and reproduction. Most approaches to alleviate the period of negative energy balance focus on mitigating the decrease in DMI and include the partial substitution of forages with more energy-dense concentrates and fat supplements (Castaneda-Gutierrez *et al*., 2005). On the other hand, pregnancy rate which is an important determination of profitability in dairy operation decrease in early lactation because of negative energy balance and low DMI (Mattos *et al*., 2002). It was shown that reproduction in ruminants is associated closely with the availability of energy (Mattos *et al*., 2000). Fat is commonly included in diets of dairy cows to increase energy density. Supplementation of fatty acids also can influence metabolic events that are important to successful reproduction of dairy cows (Burke *et al*., 1997). Fish Meal (FM) is used in dairy cow ration as a source of RUP (Mattos *et al*., 2002). Fish meal contains oil (8-10% of DM) with relatively high concentrations of 2 Polyunsaturated Fatty Acids (PUFA) of the n-3 family, Eicosapentaenoic Acid (EPA, C20:5) and Docosahexaenoic Acid (DHA, C22:6), which can be supplied only by the diet because EPA and DHA cannot be synthesized de novo

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in mammalian systems (Mattos *et al*., 2000). These 2 omega-3 fatty acids can escape ruminal biohydrogenation (Wamsley *et al*., 2005) and become incorporated in uterine endometrium (Burns *et al*., 2003). It was suggested that fatty acid, not the additional energy provided by the fatty acids, stimulate ovarian function (Petit *et al*., 2002). Supplementary fats are likely to affect fertility because fatty acids are the precursors both of Prostaglandins (PG) and via cholesterol, the steroid hormones. *In vitro* studies showed that the n-3 fatty acids EPA and DHA altered prostaglandin biosynthesis in a number of cells and tissues (Weber and Sellmayer, 1991; Mattos *et al*., 2003). The most potent COX-2 inhibitor was EPA followed by DHA (Ringbom *et al*., 2001). So, fish meal is a good source of essential amino acids and especial poly unsaturated fatty acids that could improve reproductive performance of high producing dairy cows.

The objective of this experiment was to compare diets with or without fish meal from transition period up to 35 DIM and their effects on first 60 days milk production and 305 days adjusted milk yield, follicular dynamics and complete blood count in early lactation Holstein cows.

MATERIALS AND METHODS

From approximately 3 weeks before calving to 35 days after calving, ten multiparous Holstein cows were housed in tie stalls. Cows were blocked by parity, expected calving date and previous 305 day milk production and were randomly assigned within block to 1 of 2 diets containing none (n = 5) or 3.5 and 1.95% Kilka fish meal (Mafan Co, Tehran, Iran) during the prepartum and postpartum periods, respectively. Diets were formulated to be isoenergetic and to provide similar amounts of NEL and NFC using the Cornell Net Carbohydrate and Protein System (Fox *et al*., 2004). At parturition, rations were adjusted to supply nutrients required during early lactation. Concentrate mixtures and forage sources were mixed in a weighting and mixing unit and offered in the TMR form twice daily to allow 5-10% orts (as-fed basis). Orts were collected daily and weighted. The TMR were sampled weekly throughout the experiment and DM content was determined by drying at 110°C for 18 h. Samples of TMR were collected weekly, composited monthly and analyzed by wet chemistry procedures for CP, NDF, ADF and ether extract. The weekly TMR samples were stored in -20°C until the analyses. Ingredient and nutrient composition of the diets are listed in Table 1. Cows were milked 3 times per day at 0100, 0930 and 1700 h and yields were recorded. Milk samples were collected from each milking on 1 day per week and composited for analysis of milk composition (Micro Scan; FOSS Electric A/s, Denmark). Cows were also recorded every month throughout the lactation period. Cows were weighed weekly after morning milking (0930 h). Net Energy Balance (EB) was calculated weekly from measurements of milk yield and composition, DMI, BW and NEL value of the diet using the NRC (2001) equations. The Ferdowsi University of Mashhad and Department of Animal Science Animal Care and Use Committees approved all procedures involving experimental cows.

Table 1: Ingredient and chemical composition of the experimental diets[1]

	Prepartum		Postpartum	
Variable	Control	3.5% FM	Control	1.95% FM
Ingredient, % of DM				
Corn Silage	26.81	26.81	15.64	15.64
Alfalfa Hay	17.42	17.42	21.35	21.35
Wheat - Straw	6.82	6.82	-	-
Barley Grain	10.37	10.37	10.32	10.32
Corn Grain	10.37	10.37	10.32	10.32
Molasses	-	-	3.05	3.05
Cottonseed	5.43	5.43	6.13	6.13
Soybean Meal	10.34	6.89	14.08	12.14
Beet pulp-shreds	3.48	3.48	3.13	3.13
Beet pulp-Dehydrate	-	-	1.78	1.78
Wheat Bran	6.39	6.39	10.43	10.43
Fish meal	0	3.45	0	1.94
Oystershell	0.76	0.76	0.60	0.60
Megalac	-	-	1.19	1.19
Bicarbonate sodium	-	-	0.61	0.61
Dicalcium phosphate	-	-	0.59	0.59
Ammonium Sulfate	0.46	0.46	-	-
Calcium Chloride	0.90	0.90	-	-
Magnesium Chloride	0.45	0.45	-	-
Mineral Vitamin premix[2]	-	-	0.78	0.78
Chemical analysis, % of DM				
CP	14.9	15.3	16.5	16.8
Soluble protein	32	32	28	28
peNDF	27	27	20	20
NDF	38.6	38.1	32.9	32.6
NFC	38.1	37.4	40.1	39.6
EE	3.6	3.9	4.6	4.8
Ca	1.07	1.25	0.97	1.07
P	0.37	0.45	0.55	0.60
Mg	0.24	0.23	0.28	0.28
NE_L, Mcal kg^{-1}	1.54	1.54	1.64	1.64

[1]Values represent averages of samples composited pre- and postpartum, [2]The mix (DM basis) contained: 0.57% Ca, 15.75% Sulfur, 1,362.24 ppm cobalt, 40816.32 ppm copper, 2724.49 ppm iodine, 10204.08 ppm iron, 122,448.98 ppm manganese, 122,448.98 ppm zinc

Using vacutainer tubes, blood samples were collected weekly from -7 to 35 day relative to calving via venipuncture of coccygeal vessels before the morning feeding to monitor Complete Blood Count (CBC). The blood samples for CBC were kept in room temperature until analyzing for CBC. Complete blood counts were automatically determined with a hematology analyzer (Sysmex K1000, TOA Ltd., Tokyo, Japan).

To monitor follicular parameters, ultrasound measurements of follicular activity were made on alternate days from 10-35 day to ascertain the characteristics and

fate of the 1st follicular wave, using a 7.5 MHz rectal transducer (Medison SA 600V, Seoul, Korea). Dominant follicle development was characterized by follicular mapping of recorded ultrasound images (Heravi Moussavi *et al*., 2007c). Follicular recruitment during the 1st follicular wave after parturition was evaluated by quantification of the numbers of 5-10 mm follicles on day 10 and 14 (Robinson *et al*., 2002). A dominant follicle was defined as a follicle that was >10 mm in diameter in the absence of other large (>9 mm) growing follicles.

The data repeated in time were analyzed by using a mixed model (PROC MIXED, SAS Inst. Inc., Cary, NC) for a completely randomized design with repeated measures using the following model:

$$Y_{ijk} = \mu+T_i+A_{(i)j}+D_k+(T\times D)_{ik}+\varepsilon_{ijk}$$

Where,

Y_{ijk} = Dependent variable.
μ = Overall mean.
T_i = Treatment effects.
$A_{(i)j}$ = Random effects of animal within treatments.
D_k = Effects of sampling day or time.
$(T\times D)_{ik}$ = Interaction effects of treatment and sampling day or time.
ε_{ijk} = Residual error associated with the ijk observation.

Overall effect of treatment was tested using cow within treatment as the error term. For all analyses, least squares means were calculated. Means were separated by Tukey- Kramer multiple range test.

Remaining data that were not repeated in time were analyzed by using a generalized linear model (PROC GLM; SAS Inst. Inc.) for a completely randomized design using the following model:

$$Y_{ij} = \mu+T_i+\varepsilon_{ij},$$

Where,

Y_{ij} = Dependent variable.
μ = Overall mean.
T_i = Treatment effects.
ε_{ij} = Residual error.

For analyzing the milk yields, the pervious lactation 305 days adjusted milk yield was used as a covariate. Means were separated by Duncan multiple range test.

RESULTS AND DISCUSSION

Milk production and composition and also dry matter intake were all similar among the diets (Heravi Moussavi *et al*., 2007a). First 60 days cumulative milk production and 305 days adjusted milk production were similar among diets (Table 2). Diet had no impact on energy balance during both prepartum (7.78 and 8.3±0.57 Mcal day^{-1} for Control and Supplemented groups, respectively) and postpartum periods (-3.85 and -4.25±1.3 Mcal day^{-1}, for Control and Supplemented groups, respectively). The supplemented group tended to have more negative energy balance in the postpartum period ($p = 0.09$). The effect of time was significant and energy balance decreased in the postpartum period ($p<0.05$). Our result on milk production is similar to some other reports showing no effect of feeding fish meal on milk production in early lactation dairy cows (Spain *et al*., 1995; Abu-Ghazaleh *et al*., 2001; Mattos *et al*., 2002). Reports in the literature on the effects of feeding fish meal on milk production have not been consistent. Abu-Ghazaleh *et al*. (2001) reported that substitution of soybean meal by fish meal in isonitrogenous diet basis at 0, 25, 50 and 100% of supplemental protein did not affect milk yields significantly. Several previous studies reported increased milk yield by adding fish meal (Carroll *et al*., 1994; Adachi *et al*., 2000, Heravi Moussavi *et al*., 2007b). In a review of the literature, Santos *et al*. (1998) observed that milk yield was increased by FM in 8 of 32 studies and cows producing more than 30 kg of milk per day benefited more from FM supplementation than those producing less milk per day. Amount of fish meal supplementation in diet also seems to be another variable in responding cows to

Table 2: First 60 days cumulative milk production and 305 days adjusted milk yield in cows fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

	Diets			
Parameter	Control	Supplemented	SED	p-value
First 60 days cumulative milk production, kg	2289	2295	73	0.95
305 days adjusted milk yield, kg	11003	10712	256	0.47

Table 3: Ovarian follicles and development during the first follicular wave postpartum in cows fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

	Diets			
Parameter	Control	Supplemented	SED	p-value
Number of follicles (5-10 mm in diameter) on day 10	2.6	3	0.57	0.64
Maximum diameter of first dominant follicle on day 10, mm	11.6	8.3	1.48	0.15
Maximum diameter of first dominant follicle on day 14, mm	11.1	11.9	2	0.79
Maximum diameter of first dominant follicle, mm	16.5	15.0	1.57	0.52
Number of days until dominant follicle detection	16.6	20.4	1.92	0.20
Number of days until detection of a follicle s≥10 mm in diameter	11.4	11.6	1.16	0.90

Table 4: Intervals from calving to 1st, 2nd and 3rd inseminations and days open in cows fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

	Diets			
Parameter	Control	Supplemented	SED	p-value
Interval from calving to 1st insemination, day	70.25	70.80	5.4	0.94
Interval from calving to 2nd insemination, day	93.50	108.5	21	0.65
Interval from calving to 3rd insemination, day	115.5	160.3	15.5	0.14
Days open, d	98.75	110.6	24.4	0.72

Table 5: Complete Blood Count (CBC) during the week preceding parturition in cows fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

	Diets			
Parameter	Control	Supplemented	SED	p-value
Red blood cell (RBC)	5.49	6.45	0.28	0.06
White blood cell (WBC)	7275	7433	681.00	0.86
Neutrophil	3050	3633	643.00	0.55
Lymphocyte	4131	3709	577.00	0.63
Monocytes	94	91	70.00	0.97

Table 6: Complete Blood Count (CBC) during the first five weeks postpartum in cows fed diets containing none (control) or 3.5 and 1.95% fish meal during prepartum and postpartum periods (Supplemented)

	Diets			Treat.	Time
Parameter	Control	Supplemented	SED	p-value	p-value
Red Blood Cell (RBC)	5.18	5.45	0.23	0.45	0.07
White Blood Cell (WBC)	5840	7772	713	0.10	0.11
Neutrophil	2374	4181	551	0.06	0.18
Lymphocyte	3216	3326	279	0.79	0.83
Monocytes	180	214	39	0.56	0.20

fish meal. In a recent study, it was shown that fish meal supplementation just at 5% dry matter intake increased milk production compare with the other amounts of FM supplementation (Heravi Moussavi *et al*., 2007b). Hence, the lack of any impact of fish meal on milk production in this study might be related to the amount of fish meal supplemented in the diet.

Effect of diet on follicular dynamic is shown in Table 3. Only 3 cows (2 from Control and 1 from Supplemented groups) ovulated from 10-35 day DIM. Maximum diameter of 1st dominant follicle on day 10 and 14, numbers of follicles (5-10 mm in diameter) on day 10 and 14, maximum diameter of 1st dominant follicle and number of days until dominant follicle detection were not different significantly between diets. While there is a vast array of literature on the effects of nutrition on various reproductive parameters in cattle, there is a relatively sparse literature on the specific effects of nutrition on follicle growth rate. Previous studies have demonstrated that dietary fatty acids can lead to stimulation of ovarian follicle activity (Lucy *et al*., 1991;

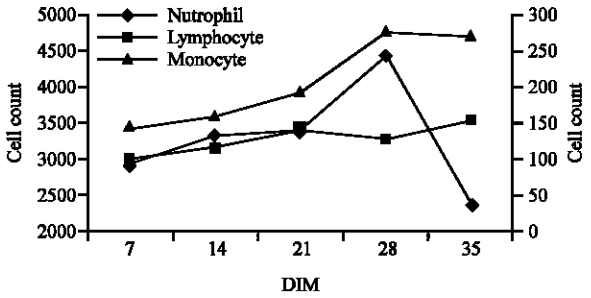

Fig. 1: Trends of neutrophil, lymphocyte and monocytes count changes during the first five weeks postpartum (pooled SEs were 536, 323 and 51, respectively)

Abayasekara and Wathes, 1999). Petit *et al*. (2002) fed omega-3 fat to Holstein dairy cows and showed that the number of class-1 follicles (3.0-4.9 mm in diameter) over time was similar among diets, but cows on the fish oil treatment tended to have a greater number of class-2 follicles (5.0-9.9 mm in diameter). Heravi Moussavi *et al*. (2007c) reported that except than the number of 5-10 mm ovarian follicles on day 10, other follicular parameters were not changed by adding FM up to 5% to diet. It seems the possible effect of fat on follicle dynamic is independent of the increased energy. It has been suggested that the enhanced follicular growth shown in some studies with saturated fats is caused by increased insulin concentrations. However, the relationships between insulin concentrations and follicular development are inconsistent, because in some studies insulin concentrations were increased, whereas in other studies, there was no change (Robinson *et al*., 2002 for review). IGF-I is another known stimulator of follicular development (Spicer and Echternkamp, 1995). According to our results it seems that the polyunsaturated fatty acid content of the supplemented diet was not sufficient to influence follicular dynamic.

Interval from calving to 1st, 2nd and 3rd inseminations were all similar among the groups (Table 4). Diet had no impact on days from calving to conception (Table 4). In agreement with our result, other study by Burke *et al*. (1997) in a large herd in Florida showed that reproductive responses, including conception rate at 1st AI, number of days open, number of AI per conception and number of days to 1st AI were unaffected by supplementing fish meal to diet. Another study by Carroll *et al*. (1994) also showed that the days to observed estrus, days to 1st AI service, conception rate, service per conception, days open and pregnancy rate were not impacted by fish meal in diet.

The effects of diet on total cell count during precalving and postcalving periods are shown in Table 5 and 6, respectively. The trends of leukocyte differential counts during the first 5 weeks postpartum are shown in Fig. 1. Diet had no impact on total cell and the differential counts both pre- and postpartum. In another study it was also shown that the diet had no effect on the blood cell counts (Doepel *et al*., 2006). In the Doepel *et al*. (2006) study the total leukocyte count was significantly increased on day 1 compared with the precalving period and then remained constant from 4-18 day. In the present study, the first sampling after parturition was within the 1st week hence we can not evaluate their funding on the difference between day 1 and precalving period on total leukocyte count but in agreement with their study we have not seen any effect of time postpartum on the differential counts.

CONCLUSION

The results of this study demonstrate that feeding fish meal pre- and postpartum had no apparent effect on first 60 days cumulative milk production and 305 days adjusted milk yield. Follicular dynamic was not impacted by the dietary groups in early lactation dairy cows. Days from calving to 1st, 2nd and 3rd inseminations and also number of days open were all similar among the dietary groups. Feeding fish meal in the present study during pre- and postpartum had no impact on immunological parameters measured as total cell count.

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