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INFLUENCE OF OMEGA – 3 RICH FATTY ACID ENRICHED FEED ON BIOCHEMICAL MARKERS AND POST PARTUM REPRODUCTIVE PERFORMANCE OF CROSS BRED DAIRY COWS

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ABSTRACT

A study was conducted to assess the influence of omega-3 rich fatty acid enriched feed on biochemical markers and postpartum reproductive performance of crossbred dairy cows. Twenty four pregnant crossbred cows were randomly divided into two groups of 12 cows each. Cows in group 1 (control) were fed with standard NRC ration plus 600g molasses while cows in group 2 (treatment) were fed with standard ration plus crushed flaxseed (omega-3 FA source) 750 g/head/day from one month before the due date of parturition till one month after parturition. Results revealed that the biochemical markers like plasma concentration of prostaglandin F metabolite (PGFM), insulin like growth factor-1 (IGF-1) and progesterone concentration were significantly ($p<0.01$) higher in feeding a source of omega-3 fatty acid as compared to control diet. Conception rate was significantly ($p<0.01$) higher (58.30 vs 33.33%) and embryo mortality was significantly ($p<0.01$) lower in supplemented cows compared to control cows (14.30 vs 50.00%). In conclusion these data demonstrated the positive effect of omega-3 fatty acid supplementation on reproductive performance. However, more studies need to be undertaken to confirm these results as well as a mechanism of action involved at the molecular level.

Key words: Conception rate, Embryonic mortality, Omega-3 FA, PGFM, Progesterone

INTRODUCTION

Supplementation of fat to pregnant cows at the end of the last trimester has become a high priority in transition cows due to the fact that there is an overwhelmingly high demand of energy for both foetal development and lactation, despite the reduced feed intake, at this stage of pregnancy. This critical period of physiological stage, termed as transition period is a period 3 weeks before and 3 weeks after parturition (Grummer, 1995). During this stage the cows exercise negative energy balance (NEB) as the onset of lactation characterized by a dramatic increase in the nutrient demands for milk synthesis that coincides with a prepartum decline in dry matter intake (DMI). Even though both saturated and unsaturated fatty acids are being used as energy sources for ruminant livestock, the extra benefit of feeding unsaturated fatty acids (USFA) over that of saturated fatty acids (SFA) is becoming the current topic of research in the globe.

Unsaturated fatty acids like linoleic and α-linolenic acid are incorporated in the arachidonic acid and eicosapentaenoic acid pathway, respectively, where the latter act as precursors of prostaglandins. But the biological activities of prostaglandin (PG) synthesized from eicosapentaenoic acid and arachidonic acid are not the same. Prostaglandins of the two series ($PGF_{2\alpha}$) are derived from arachidonic acid, whereas those of the three series ($PGF_{3\alpha}$) are formed from the eicosapentaenoic acid pathway. Dietary fatty acids from the omega-3 family (α-linolenic acid) reduce ovarian and endometrial synthesis of $PGF_{2\alpha}$, which may contribute to reduced embryonic mortality (Mattos *et al.,* 2006). This suggests that feeding omega-3 fatty acids to dairy cows would improve the fertility of cows.

On the other hand, high energy feed has also been found to increase the plasma concentration of IGF-1, which in turn improves the fertility of the cows as IGF-1 is a polypeptide hormone with anabolic properties for somatic growth and cellular metabolism (Kajimoto and Rotwein, 1989). Thus, the objective of the present study was to determine the effects of feeding a source of omega-3 fatty acid on concentration of progesterone, PGFM, IGF-1, conception rate and embryo mortality in crossbred dairy cows.

MATERIALS AND METHODS

Description of the study area

The present study was conducted at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal - Haryana, India. The NDRI, Karnal is located on 29° 43' N latitude and 76° 58' E longitudes at an altitude of 245 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. There are four major seasons in the year viz. winter (December to March), summer (April to June), rainy (July to September) and autumn (October and November). The minimum ambient temperature falls to near freezing point in winter and maximum goes approximately up to 45^{o}C in May/June months of summer. The average annual rainfall is 700 mm, most of which is received from July to September. A subtropical climate prevails in the area.

Experimental animals and treatment

Twenty four Karan Fries (KF) crossbred cows (Tharparkar X Holstein Frisian), maintained at Livestock Research Centre of National Dairy Research Institute, Karnal, Haryana, India were utilised for the study. Cows were assigned randomly to two treatments (Control-standard NRC ration for pregnant and lactating cows plus 600g molasses/day/head and treatment-standard ration plus crushed flaxseed (Omega-3 FA source) 750 g/d/head) under completely randomized design.

All cows were fed on iso nitrogenous and iso caloric rations, formulated by NDRI, so as to meet the requirements of NRC (NRC, 2001). All cows were fed *ad libitum green* fodder, provided each morning after milking. The proximate composition of feed stuffs used are given in Table 1.

Estimation of PGFM, P4 and IGF-1

For measuring hormone profile, 9 ml blood samples were collected on days 21, 14, 7 and 2 before and after calving by jugular vein puncture into heparnised (1:1000) polystyrene tubes and plasma was prepared from each sample. Immediately after the collection of blood, each blood sample was centrifuged at 4°C at the rate of 3000 rpm for 20 minutes to separate the plasma from blood cells. The separated plasma samples were stored in cryovials at -20 °C till the assay for the hormone estimation was conducted. The $PGF_2\alpha$ response was measured as the venous concentration of 13, 14-dihydro-15-keto $PGF_2\alpha$ (PGFM). Plasma PGFM was measured using the B-Bridge 13, 14-dihydro-15-keto $PGF_2\alpha$ (PGFM)

Table 1. Chemical composition of feeds, % on DM basis.

Feedstuffs	DM	CP	EE	NDF	ADF	CF	Ca	P
Maize *(Zea mays)*	17.31	8.82	2.52	57.22	37.64	28.8	0.18	0.15
Oats (Avena sativa)	15.14	10.26	1.92	47.89	29.65	26.29	0.45	0.34
Jowar *(Sorghum bicolor)*	23.00	7.64	1.76	27.81	38.42	31.42	0.39	0.19
Berseem *(Trifolium alexandrinum)*	19.00	14.6	2.98	47.00	38.9	26.96	2.32	0.40
Wheat straw	90.00	3.54	0.87	87.01	50.20	52.00	0.34	0.19
Concentrate mixture	90.00	22.01	5.33	30.82	16.10	14.63	2.81	0.63
Flaxseed	92.01	18.34	39.88	27.98	21.94	6.88	2.3	6.22

immunoassay kit (Catalog No. K3022-1 and K3022-5, B-Bridge International, Inc.).

Plasma progesterone hormone was estimated using Bovine Progesterone hormone (P_4) ELISA test Kit (Endocrine Technologies, Inc. Newark, CA). The Progesterone ELISA kit was based on the principle of solid phase enzyme-linked immunosorbent assay (Competitive binding ELISA). Plasma concentration of insulin-like growth factor - 1 (IGF - 1) was quantified by enzyme-linked immunosorbent assay kit (Cloud-Clone Corp. Uscn Life Science Inc.), a sandwich enzyme immunoassay kit for *in vitro* quantitative measurement of IGF-1 in bovine serum, plasma and other biological fluids.

Heat Detection and Insemination

Oestrus detection was carried out by visual observation. All cows were observed for signs of oestrus for 30 minutes period, twice daily and cows seen in heat in the morning were inseminated in the afternoon whereas cows seen in heat in the afternoon were inseminated in the morning.

Pregnancy Diagnosis

Around day 21 after insemination the cows were monitored for returning to heat. Non – return rate (NRR) was calculated as the difference between cows inseminated and cows seen to heat at 24 days. If animal did not return to heat after insemination, it was usually presumed as pregnant. At days 32 and 45 after AI, pregnancy was

Table 2. Least Squares Means ± SE of Plasma PGFM (ng/ml) in KF cows supplemented with Omega-3 fatty acid

Transition Period	Treatment Group	
	Omega-3 FA	Control
-21	$435.1^{dy} \pm 79.546$	$577.7^{dx} \pm 51.661$
-14	$539.7^{cy} \pm 77.259$	$856.4^{cx} \pm 155.684$
-7	$635.2^{by} \pm 124.224$	$1146.9^{bx} \pm 84.939$
-2	$690.8^{ay} \pm 92.278$	$1260.3^{ax} \pm 107.650$
+2	$320.8^{ey} \pm 192.196$	$539.9^{dx} \pm 192.189$
+7	$283.9^{fy} \pm 155.823$	$480.7^{ex} \pm 56.655$
+14	$230.3^{gy} \pm 104.729$	$371.5^{fx} \pm 135.718$
+21	$234.5^{gy} \pm 55.\ 797$	$406.5^{fx} \pm 157.449$

*means bearing different superscripts in a row; x and y and in a column; a, b, c… are significant at P<0.05

confirmed by transrectal ultrasonography and by palpation after 50 days. Cows were monitored for return to oestrus and bred again if detected in oestrus.

Embryonic Mortality and Conception rate

Conception rate was defined as the proportion of cows that were detected in oestrus and inseminated to that which were pregnant on day 32 post A.I. Embryo mortality was calculated as the difference between the number of cows that were pregnant on day 32 and that were pregnant on day 45.

Statistical analysis

Data were subjected to t-test and chi-square test for CRD using the SAS (SAS institute, 2002) for repeat measures. Graphic pad PRISM was used to analysis both in grouped and or in column wise for lines and graph comparisons. Significance was declared at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.1$, unless otherwise stated. When a significant F-test was detected, multiple comparisons were made to discriminate among the means using Tukey's honestly significant difference (HSD) procedure.

RESULTS AND DISCUSSION

Plasma concentrations of PGFM, P4 and IGF-1

The mean plasma concentration of 13, 14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) during the transition period is presented in Table 2. The concentrations of PGFM significantly differed between treatment groups (P<0.01) as well as during different days in transition period (P<0.05). Flaxseed supplemented cows

Table 3. Least Squares Means ± SE of Plasma Progesterone (ng/ml) in KF cows supplemented with Omega-3 fatty acid

Transition Period	Treatment Group	
	Omega-3 FA	Control
-21	$5.230^{ax} \pm 0.969$	$5.24^{ax} \pm 0.635$
-14	$5.430^{ax} \pm 0.603$	$4.460^{by} \pm 0.333$
-7	$5.404^{ax} \pm 0.820$	$4.380^{by} \pm 0.333$
-2	$2.192^{bx} \pm 0.283$	$1.239^{cy} \pm 0.383$
+2	$0.532^{dx} \pm 0.102$	$0.394^{dx} \pm 0.141$
+7	$0.670^{cdx} \pm 0.104$	$0.575^{dx} \pm 0.137$
+14	$0.764^{cdx} \pm 0.337$	$0.458^{dx} \pm 0.111$
+21	$1.195^{cx} \pm 0.143$	$0.610^{dy} \pm 0.116$

*means bearing different superscripts in a row; x and y and in a column; a, b, c… are significant at P<0.05

had significantly lower concentrations of PGFM. The trend analysis revealed that the concentration of PGFM reached peak two days before parturition. Plasma concentrations of PGFM were found to be significantly reduced in α-linolenic acid (omega-3 FA) supplemented cows as compared to control cows. The present finding is in agreement with Fuentes *et al*. (2008) and Nazir *et al*. (2012) who reported lower mean plasma PGFM concentration in flaxseed supplemented groups of dairy cows and Murrah buffalo, respectively. This may be because α-linolenic acid (omega-3 FA) present in the flaxseed can inhibit prostaglandin synthesis by competitive inhibition of cyclooxygenase enzymes. The α-linolenic acid (C18:3n-3) is a precursor to eicosapentaenoic acid (EPA, C20:5n-3) and linoleic acid is a precursor to docosahexaenoic acid (DHA; C22:6n-3).

Both EPA and DHA interfere with the conversion of arachidonic acid to $PGF_{2\alpha}$ (Binelli *et al*., 2001). Regulatory enzymes for this conversion include delta 6 desaturase and cyclooxygenase. It is important to note that linolenic acid (C18:3) was also present in the endometrial prostaglandin synthesis inhibitor isolated by Thatcher *et al*. (1994) and has been shown to be a strong inhibitor of prostaglandin synthesis (Mattos *et al*., 2000), which has been suggested to have implications in embryonic survival (Thatcher *et al*., 2001; Santos *et al*., 2004).

The mean value of plasma progesterone concentrations was significantly ($P<0.05$) higher in flaxseed supplemented cows (2.677 ± 0.804) compared to control cows (2.171± 0.750). Moreover, when data of plasma progesterone concentrations were analysed from day 21 before parturition to day 21 postpartum, significantly ($p<0.05$) higher levels of progesterone was observed in cows fed on omega-3 FA source diet compared to control cows at all levels, except at day 2, 7 and 14 after parturition. High plasma progesterone concentration in flaxseed supplemented group of cows indicated that corpus luteum was likely to be less affected by $PGF_{2\alpha}$ in this group as compared to the control cows. In agreement to the current study, higher serum concentration of progesterone was reported for flaxseed supplemented cows than either Megalac or soybean supplementation (Petit and Twagiramungu, 2006).

Similarly, Burke *et al*. (1997) reported larger proportion of cows at AI with plasma progesterone concentration greater than 1 ng per ml when fish meal, which is high in omega-3 fatty acid, was included in the diet than the unsupplemented control. The value recorded during the present study was comparable to the value

(2.80 ± 0.92 ng/ml) reported by Mulugeta *et al*. (2004) on postpartum serum progesterone concentration of Horro cattle breed in Ethiopia during dry season, but lower than the value (8.50 ± 0.80 ng/ml) reported during the wet season in Horro cows.

Suppression of $PGF_{2\alpha,}$ secretion and maintenance of the CL are obligatory steps for establishment of pregnancy of cows (Tatcher *et al*., 1994). In this regard, Mattos *et al*. (2000) suggested the use of fish meal due to high omega-3 fatty acid content. Hence, in the present study, omega-3 fatty acid contained in flaxseed could have reduced the sensitivity of the corpus luteum to $PGF_{2\alpha}$ or reduced the uterine secretion of $PGF_{2\alpha}$ that delayed the completion of functional luteolysis which might have resulted in incomplete corpus luteum regression.

The trend for a higher peak value of progesterone concentration for cows fed flaxseed compared to control was consistent with the positive effect of omega-3 fatty acids on progesterone concentration. This could probably be due to the fact that feeding omega-3 fatty acids might induce granulose cell proliferation and increase follicular size as reported for cows fed high-fat diets (Petit and Twagiramungu, 2006), which would result in larger CL and stimulate ovarian steroidogenesis and progesterone secretion.

The least square means of plasma concentration of IGF-1 in KF cows supplemented with or without omega-3 FA source feed has been presented in Table 4.

Table 4. Least Squares Means ± SE of Plasma IGF-1 (pg/ml) in KF cows fed on diets supplemented with Omega-3 fatty acid

Transition Period	Treatment Group	
	Omega-3 FA	Control
-21	$49.687^{cx} \pm 2.261$	$44.302^{ay} \pm 3.085$
-14	$50.255^{bcx} \pm 2.181$	$40.795^{aby} \pm 2.192$
-7	$47.538^{cx} \pm 3.880$	$36.302^{cdey} \pm 4.099$
-2	$41.965^{dx} \pm 2.447$	$32.775^{dey} \pm 2.556$
+2	$40.656^{dx} \pm 3.481$	$32.240^{ey} \pm 3.547$
+7	$46.634^{cx} \pm 5.458$	$37.136^{bcdy} \pm 6.388$
+14	$58.626^{ax} \pm 1.408$	$41.464^{aby} \pm 4.367$
+21	$54.203^{bx} \pm 5.298$	$39.773^{bcy} \pm 5.876$

*means bearing different superscripts in a row; x and y and in a column; a, b, c… are significant at $P<0.05$; LSM-least square means; SE-standard error;

Results of the study indicated that there was significantly higher (P<0.05) IGF-1 concentration in cows supplemented with omega-3 FA compared to control cows. It was also noticed that plasma concentration of IGF-1 was generally higher in prepartum than postpartum period. The present finding is at par with Abribat *et al.,* (1990) who observed lowest serum IGF-1 concentration in dairy cows after 24 hour of parturition (45 ng/mL), which subsequently increased and remained at a higher level throughout the eight months of lactation (90 ng per ml) increasing further to 110 ng per ml during the dry period.

Since IGF-1 is a polypeptide hormone with anabolic properties for somatic growth and cellular metabolism, greater conception rate was observed from the first and in all artificial inseminations done in cows with higher IGF-1 concentrations. This result is corroborated by Falkenberg *et al*. (2008), who observed higher conception rates in cows with IGF-1 concentrations above the median, compared with cows with IGF-1 concentrations, below the median.

Conception Rate, NSPC, Days open and Embryo Mortality

The conception rate (%), number of services per conception, days open and embryo mortality in control and treatment groups are presented in Table 5. The conception rate in cows to first service was significantly higher (58.30%) in omega-3 FA supplemented group (p<0.01) compared to those cows in control group (33.33%). The number of services per conception in cows fed on diets supplemented with omega -3 FA (1.33) was significantly (p<0.01) in cows fed on control diet (1.67). The overall mean for days open was 86.17 ± 9.51 and 168.83 ± 26.75 in omega–3 FA supplemented and control cows, respectively (Table 5). Treatment had significant effect on days open (P<0.05). More importantly, when the data was analysed day wise, only one cow (8.30%) conceived within 100 days after parturition in control group as against 41.70% (n=5) cows from omega-3 FA supplemented group. It was also observed that cows fed on diet supplemented with omega-3 rich FA completed involution by 30 days, returned to cyclicity and bred earlier than the control cows. Moreover, larger size of corpus luteum and subsequently, higher progesterone production was reported for omega-3 FA supplemented as compared to control cows (Ulfina *et al*., 2015) for the same herd.

The present study clearly indicated the usefulness of flaxseed supplementation in dairy cows for attenuating the luteolytic signal ($PGF_{2\alpha}$) during the period of pregnancy recognition, improving the post-breeding luteal profile progesterone and hence enhancing the conception rate. The present findings were at par with that of Petit *et al*. (2001) and Ambrose *et al*. (2002, 2006) who reported significant improvements in pregnancy rates in cows fed a flaxseed supplemented ration Similar trend was reported (Nazir *et al*., 2013) on flaxseed supplemented buffalo (66.7%) in comparison to non-supplemented buffalo (31.20%.)

Contrary results also exist with no improvement in conception rate in dairy cattle following supplementation with extruded, rolled or whole flaxseed (Petit and Twagiramungu, 2006; Fuentes *et al*., 2008; Bork *et al*., 2010). The positive impact of flaxseed supplementation on pregnancy rate in dairy cattle may be attributed to higher IGF-1, increased plasma progesterone and suppression of $PGF_{2\alpha}$ release. The result confirms the hypothesis that feeding flaxseed would increase conception rate of dairy cows due to a decrease in PGFM concentration and thereby its luteolytic effect.

Total embryo mortality was significantly ($p<0.01$) lower for cows supplemented with omega-3 FA (14.30%) as compared to cows (50.0%) in control group. The results clearly indicate that embryo mortality or pregnancy loss was significantly reduced by feeding α-linolenic acid during transition period in dairy cattle. This would agree with Juchem *et al*. (2002) who reported that although conception rate at day 28 after AI was similar for cows fed calcium salts of palm and fish oils and those fed tallow (42.60% vs 40.70%), pregnancy loss from day 28 to 39 after AI was reduced for cows fed the former diet (0 vs 15%, $p<0.10$). In their work on conception rate and reproductive function of dairy cows fed different fat sources, Petit and Twagiramungu (2006) also observed similar trends for the cows fed on megalac® and micronized soybean. (calcium salts of palm fatty acids)

The decrease in embryo mortality in cows fed with omega - 3 FA source may be the result of different types of PGs being synthesized. The present finding was corroborated by Ambrose *et al*. (2002) who reported lower biological activity in the trienoic PG ($PGF_3\alpha$) than the corresponding dienoic PG ($PGF_2\alpha$), which would contribute to improve fertility. Decreased concentration of $PGF_2\alpha$ during the breeding period has been reported for cows fed on-omega-3 fatty acids compared to those fed on omega-6 fatty acids or calcium salts of palm oil (Lassard *et al*., 2003). Moreover, the benefit of omega-3 fatty acids contained in flaxseed have resulted from its supplementary effect that could have reduced the sensitivity of the

Table 5. Post partum reproductive performance of KF cows supplemented with Omega-3 fatty acid

Parameters	Treatment group	
	Omega-3 FA	Control
DFOH, d	43.60± 3.85[b]	76.80 ±10.08[a]
DFS, d	72.20 ± 6.24[b]	116.00 ± 10.20[a]
CR 3 A.I, %	58.30[a]	33.33[b]
EM, %	14.30[b]	50.00[a]
NSPC, No.	1.33 [b] ± 0.120	1.67[a] ± 0.130
DO, d	86.17[b]± 9.51	168.83[a]± 26.75

*means bearing different superscripts in a row are significant at $P<0.05$
(DFOH-days to 1st observed heat; DFS-days to 1st service, CR-conception rate, A.I.-artificial insemination, EM-embryo mortality, NSPC-number of services per conception, Days open-days open, d-day, No.-number, FA-fatty acid, KF-Karan fries)

corpus luteum to $PGF_2\alpha$. In agreement to the current study, Thatcher *et al*. (1994) had elucidated that the suppression of PGF2α concentration and maintenance of the corpus luteum are obligatory for establishment of pregnancy of cows. Petit and Twagiramungu (2006) have also indicated that changes in luteolysis during the period of maternal recognition of pregnancy could also contribute to increase embryonic survival beyond day 28. Ambrose *et al*. (2006) also observed lower pregnancy losses in cows fed flaxseed (9.8%) compared with those fed sunflower (27.30%).

SUMMARY

Results clearly indicate that inclusion of omega-3 rich fatty acid source, flaxseed in transition cow's diet resulted in lowered concentration of PGFM, increased IGF-1 and progesterone concentrations, improved conception rates and reduced pregnancy losses. It is therefore imperative to note that unsaturated fatty acid could play a multiple role due to their fatty acid composition. In conclusion, these data demonstrated the positive effect of omega-3 fatty acid supplementation on biochemical markers, thereby modulating conception rate and reducing embryo mortality. However, comprehensive studies need to be undertaken to confirm these results as well as the mechanism of action involved at molecular level.

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