Changes in Hormonal Profile, Uterine Involution and Postpartum Reproductive Performance of Crossbred Dairy Cows Supplemented with Omega–3 Fatty Acid Rich Feed during Transition Period

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Abstract: The study was conducted to determine the effects of fat (α-Linolenic acid) supplementation during transition period on postpartum reproductive performance in crossbred dairy cattle. It was hypothesized that feeding flaxseed, which is a source rich in omega-3 fatty acids (FA) would improve the postpartum reproductive performance of crossbred dairy cattle. Advanced pregnant crossbred cows (n = 24) were divided into two groups of 12 cows each. Cows in group 1 (control) were fed with standard ration plus 600g molasses/day/head based on National Research Council recommendation, 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. The parameters studied were uterine involution status, resumption of ovarian activity, progesterone concentration, conception rate, embryo mortality, plasma concentration of prostaglandin F metabolite (PGFM) and insulin like growth factor-1 (IGF-1). Compared to cows only receiving standard ration, flaxseed supplemented cows showed lower ($p<0.01$) average mean (3.48 ± 0.179ng/ml) PGFM but higher average (3.48 ± 0.179 pg/ml), P4 and (46.695 ±1.491 pg/ml) IGF-1 concentration at across different Transition Period sampling dates. Earlier uterine involution and resumption of ovarian activity ($p<0.05$) was observed in flaxseed supplemented groups compared to control ones. Conception rate was also significantly ($p<0.01$) higher and embryo mortality was significantly ($p<0.01$) lower in supplemented cows compared with control cows. It could be inferred from the study that the postpartum reproductive performance of cattle can be improved through supplementation of α-linolenic acid during transition period.

Keywords: *Conception rate, Embryonic mortality, Omega-3 FA, PGFM, Uterine involution*

Introduction

The transition period for dairy cows, defined as 3 weeks pre-calving to 3 weeks post-calving is the most critical 6 weeks of the production cycle (Grummer, 1995) and has become a focal point for research world over during the last two decades. During this critical period, the cow's metabolism shifts from the demands of pregnancy to those of lactation, with overwhelming increased demands for energy which would not be met with conventional energy sources for ruminants. This results in negative energy balance, especially in early lactation. Supplementation of fat to pregnant cows at the end of the last trimester is recommended to combat the overwhelmingly high demand for energy for both foetus development and lactation, in the face of the reduced feed intake, at this stage of pregnancy. During this stage the ruminant animals experience negative energy balance (NEB) as the onset of lactation characterized by a dramatic increase in the nutrient demands for milk synthesis 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 around the globe (Gulliver *et al*., 2012).

Unsaturated fatty acids like linoleic and α-linolenic acid are incorporated in the arachidonic acid and eicosapentaenoic acid pathway, respectively, where the latter are precursors of prostaglandins (Petit and Twagiramungu, 2006). 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.,* 2000), suggesting that feeding omega-3 fatty acids to dairy cows would improve the fertility of cows. High energy feed has also been found to increase the plasma concentration of insulin like growth factor-1 (IGF-1), which is known to improve the fertility of cows as IGF-1 is a polypeptide hormone with anabolic properties for somatic growth and cellular metabolism (Kajimoto and Rotwein, 1989). Several reports were available for the effect of feeding PUFA which is source of omega-3 fatty acid and its effect on post-partum reproductive performance. For example,

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Heravi *et al.* (2007) and Bilby *et al.* (2006) reported that dairy cows fed increased amounts of fish meal or Ca salts of fish oil, two sources of omega-3 FAs, had lower endometrial concentrations of arachidonic acid and increased concentrations of eicosapentaenoic acid and total omega-3 FA. Dirandeh *et al.* (2013) reported that mean plasma progesterone concentration was higher in cows fed flaxseed than in those fed palm oil; which indicates the suppression of $PGF_{2\alpha}$ and its lutolytic effect that results in less embryonic mortality and more pregnancy rate. However, information on the effect omega-3 rich source flaxseed on the post-partum reproductive performance of KF cows is scanty. Thus, the objective of the study was to evaluate benefits of α-linolenic acid rich flaxseed supplementation to standard transition feed of dairy cattle for improving postpartum reproductive efficiency of crossbred K-F cows at NDRI –Karnaal.

Materials and Methods

Description of the Study Area

The study was conducted at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal-Haryana, India during 2014 to 2015. The NDRI, Karnal is located on 29^0 43' N latitude and 76^0 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^oC 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 (24) Karan Fries (KF) crossbred cows (Tharparkar X Holstein Frisian), maintained at the Livestock Research Centre of National dairy Research Institute, Karnal-Haryana state, India were utilized for the study. Cows in their 2nd and 3rd parity with similar body condition were selected according to their expected producing ability (EPA) calculated based on their previous lactation milk yield. Accordingly, the EPA for the selected cows was >2000 kg/305 day lactation milk yield. Then 12 cows were allotted to each of the two treatment groups (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/day/head) under completely randomized design (CRD) assuming that all the selected cows homogenous in milk production potential (EPA, parity and body conditions.

All cows were fed on isoenergetic diet formulated as per the recommendation of NDRI to meet or exceeded the predicted requirements of National Research Council (NRC, 2001). All cows were fed *ad libitum* with fresh fodder provided each morning after milking.

Estimation of Prostaglandin F Metabolite (PGFM), Progesterone (P4) and Insulin Like Growth Factor 1 (IGF-1)

For measuring hormone profile, 9 ml blood samples were collected on the days 21, 14, 7 and 2 before and after calving (calving = day 0) by jugular venipuncture into heparnized (1:1000) polystyrene tubes; and plasma was extracted from each sample. Immediately after the collection of blood, each blood sample was centrifuged at 4^0C at the rate of 3000 rpm for 20 minutes to separate the plasma. The separated plasma samples were stored in cryovials at -20 ^0C 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). In most species $PGF_2\alpha$ is rapidly metabolized through the pulmonary system (Weems *et al*., 2006), hence plasma concentrations of its more stable metabolite (PGFM) was measured. Plasma PGFM was measured using the B-Bridge 13, 14-dihydro-15-keto $PGF_2\alpha$ (PGFM) immunoassay kit (catalog no K3022-1 and K3022-5, B-Bridge international, Inc.).

Plasma progesterone level 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 ELISA kit (Cloud-Clone Corp. Uscn life Science Inc.USA). The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of IGF-1in bovine serum, plasma and other biological fluids.

Evaluation of Uterine Involution

For uterine involution, the organs were palpated per rectum at 10, 20 and 30 days postpartum. The diameters of the organs were measured by subjectively estimating the thickness using the fingers to the nearest half centimetre (Keirse*,* 2011). Complete involution was declared when the diameter of the erstwhile gravid uterine horn and cervix had reduced to the least possible values, when no further changes in diameter could be differentiated during two successive examinations.

Estrus Detection and Artificial Insemination

Estrus detection was carried out by visual observation. All cows were observed for signs of oestrus starting 30 days for six months after calving two times daily for 30 minutes 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, assuming that maximum fertility to Artificial Insemination (AI) occurs when the cow bred at the standing heat which could take place between 6 to 12h after the cow seen in heat.

Pregnancy Diagnosis

Three methods of pregnancy diagnosis (PD) were employed. Around day 21 after insemination, the cows were monitored for returning to heat. Non–return rate (NRR) was calculated as the proportion of cows inseminated and cows didn't come to heat within 24 days. If animal is not returned to heat after insemination, it was presumed as pregnant. At day 30 (plate 1) and day 45 (plate 2) after AI, pregnancy was confirmed by transrectal ultrasonography and by palpation after 50 days. Cows were monitored for six month after postpartum for return to oestrus and bred again if detected in oestrus.

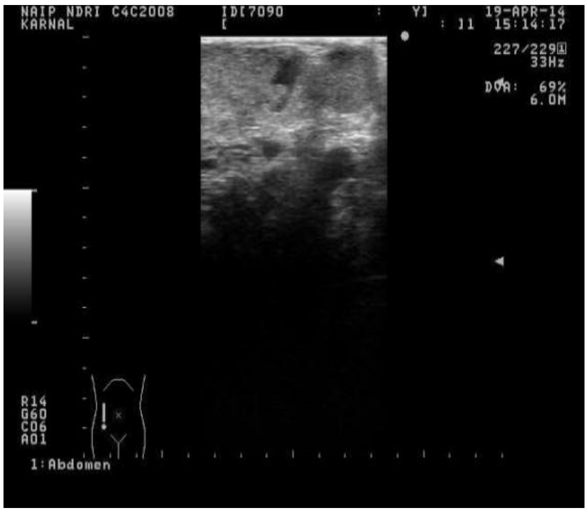

Plate 1. Ultrasonography of PD at 30 d post AI.

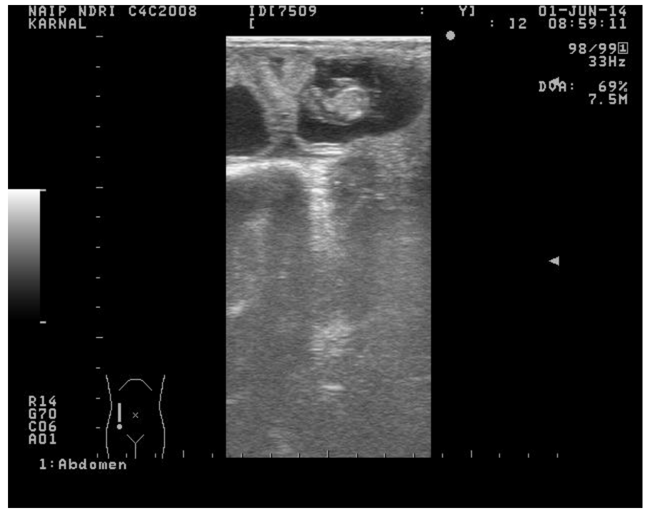

Plate 2. Ultrasonography of PD at 45 d post AI.

Conception Rate and Embryonic Mortality

Conception rate was calculated as the proportion of cows that were pregnant on day 30 post AI to that were detected in oestrus and inseminated. The first, second and third AI, if any, were considered as total conception rate to 3 AI. Because beyond three successful AI the cows were regarded as problem cow or repeat breeder. Embryo mortality was calculated as the proportion of the difference between the numbers of cows that were pregnant on day 45 post AI to the number of cows that were pregnant on day 30 post AI.

Statistical Analysis

Data were subjected to t-test for Completely Randomized Design using the SAS (SAS institute, 2002) for repeated measures. Graphic pad PRISM was used to analysis both in grouped and/or in column wise for the entire transition period. Significance was declared at $P \leq 0.05$. When a significance was detected, multiple comparisons were made to discriminate among the means using Tukey's honestly significant difference (HSD) procedure.

Results

Plasma Concentrations of PGFM

The mean plasma concentration of 13, 14-dihydro-15-keto-prostaglandin $F_2\alpha$ (PGFM) during the transition period is presented in Table 1. The concentrations of PGFM significantly differed between treatment group ($P<0.01$) as well as during different days in transition period ($P<0.05$). Flaxseed supplemented cows had significantly lower concentrations of PGFM (438.5 ± 81.71pg/ml) compared with those cows fed on control diet (750.7 ± 5.85pg/ml). The concentration of PGFM reached its peak 2 days before parturition.

Table 1. Concentration (Mean ± SE) of plasma PGFM in study animals.

Transition Period (days)	Treatment Group	
	Omega-3 FA fed cows (pg/ml)	Control cows (pg/ml)
-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	$234.5^{gy} \pm 55.797$	$406.5^{fx} \pm 157.449$
Overall Mean	$438.5^{z} \pm 81.71$	$750.7^{x} \pm 50.8$

Mean values bearing different superscripts in a row; x and y and in a column; a, b, c are significantly different at P<0.05.

Plasma Concentrations of Progesterone (P4)

The mean value of plasma progesterone concentrations determined during transition period was significantly ($P<0.05$) higher in flaxseed supplemented cows (3.48 ± 0.179) compared to control cows (2.242 ± 0.252). Moreover, when data of plasma progesterone concentrations were analyzed from day 21 before parturition to day 21 postpartum, at all levels significantly ($p<0.05$) higher levels of progesterone was observed in cows fed on mega-3 FA rich feed supplemented diet compared to control cows, except at day 2, 7 and 14 after parturition.

Table 2. Concentration (Mean ± SE) of plasma progesterone in study animals.

Transition Period (days)	Treatment Group	
	Omega-3 FA fed cows (ng/ml)	Control cows (ng/ml)
-21	5.230^{ax} ± 0.969	5.24^{ax} ± 0.635
-14	5.430^{ax} ± 0.603	4.460^{by} ± 0.333
-7	5.404^{ax} ± 0.820	4.380^{by} ± 0.333
-2	2.192^{bx} ± 0.283	1.239^{cy} ± 0.383
+2	0.532^{dx} ± 0.102	0.394^{dx} ± 0.141
+7	0.670^{cdx} ± 0.104	0.575^{dx} ± 0.137
+14	0.764^{cdx} ± 0.337	0.458^{dx} ± 0.111
+21	1.195^{cx} ± 0.143	0.610^{dy} ± 0.116
Overall Mean	3.48^{x} ± 0.179	2.242^{y} ± 0.252

Mean values bearing different superscripts in a row; x and y and in a column; a, b, c are significantly different at P<0.05

Plasma Concentrations of IGF-1

The least square means of plasma concentration of IGF-1 in KF cows supplemented with or without flaxseed is presented in Table 3. Results of the study indicated that there was significantly higher ($P<0.05$) IGF-1 concentration in cows supplemented with flaxseed compared to control cows. It was also noticed that plasma concentration of IGF-1 was generally higher in prepartum period than postpartum period.

Table 3. Concentration (Mean ± SE) of plasma IGF-1 in study animals.

Transition Period (days)	Treatment Group	
	Omega-3 FA fed cows (pg/ml)	Control cows (pg/ml)
-21	49.687cx ± 2.261	44.302 ay ± 3.085
-14	50.255^{bcx} ± 2.181	40.795^{aby} ± 2.192
-7	47.538^{cx} ± 3.880	36.302^{cdey} ± 4.099
-2	41.965^{dx} ± 2.447	32.775^{dey} ± 2.556
+2	40.656^{dx} ± 3.481	32.240^{ey} ± 3.547
+7	46.634^{cx} ± 5.458	37.136^{bcdy} ± 6.388
+14	58.626^{ax} ± 1.408	41.464^{aby} ± 4.367
+21	54.203^{bx} ± 5.298	39.773^{bcy} ± 5.876
Overall mean	**46.695^{x} ±1.491**	**38.098^{y} ± 1.137**

Mean values 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; DTP= During transition period.

Uterine Involution and Subsequent Reproductive Performance

Cows in flaxseed supplemented group completed uterine involution within 30 days, which was significantly shorter than that of the control group of cows ($p<0.05$) (Table 4). Days to first service (AI) was significantly shorter (72.2 ± 6.24) for flaxseed supplemented cows than those cows in the control group (116.0 ± 10.200) ($P<0.05$).

Table 4. Postpartum reproductive performance of study animals.

Parameters	Treatment group	
	Omega-3 FA fed cows	Control cows
Uterine involution by Day 30 (%)	100^{a}	61.5^{b}
Days to First Observed Heat (d)	43.6 ± 3.85^{b}	76.8 ± 10.08^{a}
Days to First Service (d)	72.2 ± 6.24^{b}	116.0 ± 10.20^{a}
Conception Rate to 3 A.I. (%)	58.3^{a}	33.33^{b}
Embryo Mortality (%)	14.3^{b}	50.0^{a}
NSPC (No)	1.33^{b} ± 0.120	1.67^{a} ± 0.130
Days Open (d)	86.17^{b} ± 9.51	168.83^{a} ± 26.75

Mean values bearing different superscripts in a row are significant at P<0.05.

The conception rate (%), NSPC, days open and embryo mortality in control and treatment groups is presented in Table 4. The conception rate in cows to first service was significantly higher (58.3%) in omega-3 FA source feed supplemented group ($p<0.01$) compared to those cows in control group (33.3%). The number of services per conception in cows fed on diets supplemented with omega-3 FA source feed (1.33), was significantly ($p<0.01$) lower than cows fed on control diet (1.67). The overall mean for days open was 86.17 ± 9.5 and 168.3 ± 26.75 in omega –3 FA supplemented and control cows, respectively ($P<0.05$). It was observed that total embryo mortality was significantly ($p<0.01$) lower for cows supplemented with omega-3 FA (14.3%) as compared to cows (50.0%) in control group.

Discussion

Plasma Concentrations of PGFM, Progesterone (P4) and IGF-1

Plasma concentrations of PGFM were found to be significantly reduced in omega-3 fatty acid source feed supplemented cows as compared to control cows. The present finding is in agreement with Fuentes *et al.* (2008) and Nazir *et al.* (2013) 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 different actions including competition with linoleic acid (omega-6 FA) for binding with delta 6 desaturase. 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 present in the endometrial prostaglandin synthesis inhibitor isolated by Thatcher *et al.* (1994), and has been shown to be a strong inhibitor of prostaglandin (2-series) synthesis which has been suggested to have positive implications in embryonic survival, because treatments that reduce ovarian and endometrial synthesis of $PGF_{2\alpha}$, at the expense of $PGF_{3\alpha}$, may contribute to a reduction in embryonic mortality (Mattos *et al.*, 2000).

Plasma progesterone concentration was at high in flaxseed supplemented group of cows as corpus luteum was likely less influenced 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 (Petit *et al.*, 2001) or soybean supplementation (Petit and Twagiramungu, 2006). The range of values reported in this study is similar to Muhammed *et al.* (2000), who observed plasma progesterone concentrations of 2.3 - 4.0 ng/ml in pregnant and 0.1 - 2.2 ng/ml in non pregnant cows. The current study is consistent to Nazir *et al.* (2013) who reported highly significant plasma progesterone level during supplementation as compared post supplementation of flaxseed in Murrah buffaloes.

Similarly, Burke *et al.* (1997) reported larger proportion of cows at AI with plasma progesterone concentration greater than 1 ng/ml when fish meal, which is high in omega-3 fatty acid, was included in the diet than when a control diet was fed. The value recorded during the present study was also similar to the value (2.8 ± 0.92ng/ml) reported by Mulugeta *et al.* (2004) on postpartum serum progesterone concentration of Horro cattle breed in Ethiopia during dry season, but by far lower than the value (8.5 ± 0.8ng/ml) reported during the wet season in Horro cows. As suggested by Mattos *et al.* (2000) for fish meal, omega-3 fatty acid contained in flaxseed increases progesterone concentrations in the blood by reducing the sensitivity of the corpus luteum to $PGF_{2\alpha}$ or by reducing the uterine secretion of $PGF_{2\alpha}$ that delayed the completion of functional luteolysis, which results in incomplete corpus luteum regression. In this respect, Thatcher *et al.* (1994) indicated the suppression of $PGF_{2\alpha}$, secretion and maintenance of the corpus luteum (CL) are obligatory steps for establishment of pregnancy of cows. 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 is due to the fact that feeding omega-3 fatty acids might induce granulosa 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 analysis of IGF-1 showed significantly higher concentration of IGF-1 in supplemented group. Similarly, the addition of Omega-3 fatty acids to a low fat diet increases IGF-1 in human (Young *et al.,* 2013). The present finding is at par with Abribat *et al.* (1990) who observed lowest serum IGF-1 concentration in dairy cows after 24 h of parturition (45 ng/mL), and then increased and finally remained at a higher level throughout the 8 months of lactation (90 ng/mL), increasing further to 110 ng/ml during the dry period. Likewise, Nikolic *et al.* (2001) also reported that the lowest serum IGF-1 concentration (39 ng/mL) in female cattle was found in post parturient Holstein cow and the highest (157 ng/mL) was in the growing beef heifers.

Uterine Involution and Subsequent Reproductive Performance

Uterine involution is a natural process that involves the uterus returning to its pre-pregnancy state which is a significant change after parturition that allows a cow to regain comfort and fertility. Postpartum management and parturition condition largely affects the speed of shrinkage of uterine to its normal position or returns to the way it was before pregnancy and uterine involution is a secondary indicator of postpartum reproductive performance. In the present study an early cervical and uterine involution ($P < 0.01$) in supplemented group of cows as compared to those cows in control group (Table 4). The observation that crossbred supplemented cows exhibited early cervical and uterine involution in comparison to control cows indicate that better immunity in the supplemented cows ensured greater protection against infections and hence faster recovery and early involution. Perusal of Table 4, indicated that all the cows (100%) from flaxseed supplemented group completed uterine involution before 30 days against 61.5% for the non-supplemented cows. This is because of the anti-inflammatory property and enhancement of immune process ensured by omega-3 FA source of flaxseed. This was further proved by enhanced days to first service (AI) which is significantly shorter ($P<0.05$) for flaxseed supplemented cows (72.2 ± 6.24) than those cows in control group (116.0 ± 10.200). Beyond its anti-inflammatory property, omega-3 FA (flaxseed) has a potent advantage of enhancing postpartum performance by attenuation of $PGF_{2\alpha}$ and thereby increasing corpus luteum size for increased progesterone production. α-Linolenic acid (ALA), an omega-3 fatty acid, present in flaxseed suppresses $PGF_{2\alpha}$ synthesis by inhibiting the endometrial expression of cyclooxygenase-2 (COX- 2), a rate limiting enzyme for $PGF_{2\alpha}$ synthesis (Palin *et al*., 2005). COX-2 is also an enzyme responsible for inflammation and pain. Moreover, ALA inhibits $PGF_{2\alpha}$ release through decreased availability of arachidonic acid and through increased competition of ALA with arachidonic acid for binding to COX-2 (Mattos *et al*., 2000).

It was noticed from the results that cows supplemented with omega-3 FA (crushed flaxseed) had a significantly higher conception rate over the control cows. More importantly when we analysed the data day wise, only 1 cow (8.3%) conceived within 100 days after parturition in control group against 41.7% (n=5) cows from omega-3 FA supplemented group. In our previous work we also observed cows fed on diet supplemented with omega-3 FA completed involution by day 30 postpartum, returned to cyclicity and bred earlier than the control cows. Similar trend was reported (Nazir *et al*., 2013) on flaxseed supplemented buffaloes (66.7%) in comparison to non-supplemented ones (31.2%). The present study indicates 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) who reported significant improvements in pregnancy rates in cows fed a flaxseed supplemented ration. This result is corroborated by Ambrose *et al.* (2006) who reported that supplementation of rolled flaxseed for 60 days increased conception rate in cows. Other studies also observed a positive impact of dietary inclusion of flaxseed on pregnancy rate in cattle (Petit *et al*., 2001; Petit and Berthiaume, 2006). Similarly, supplementation of crushed flaxseed to buffaloes at 15% DM for 60 days (Nazir *et al*., 2013) exhibited a 35.5 % increase in conception rate. Contrary results also exist with no improvement in conception rate in dairy cattle following supplementation with extruded, rolled or whole flaxseed (Petii 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, ovulation of large follicle, improved CL diameter and 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 $PGF_2\alpha$ production and thereby its lutyleotic effect. In addition, IGF-1 is a polypeptide hormone with anabolic properties for somatic growth and cellular metabolism we observed greater conception rate to first and all artificial insemination 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.

The results clearly indicate that embryo mortality or pregnancy loss was significantly reduced by feeding α-linolenic acid source feed 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 (42.6% vs 40.7%), 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 megalaec® and micronized soybean. In another study, Burke *et al.* (1997) reported the tendency for improvement of conception rate by 120 day postpartum ($p<0.06$) by feeding fish meal (another source of Omega-3 FA) at one dairy (31.9% vs. 41.3%) while there was no improvement at another dairy with better fertility (65.4% vs. 60.2); indicating that feeding omega-3 fatty acids may improve embryo survival in herds with lower fertility.

The decrease in embryo mortality in cows fed with omega - 3 FA source may be the result of the trienoic PG ($PGF_3\alpha$) being synthesized instead of $PGF_2\alpha$. Decreased concentration of $PGF_2\alpha$ during the breeding period has been reported for cows fed with omega-3 fatty acids compared to those fed with omega-6 fatty acids or calcium salts of palm oil (Lassard *et al*., 2003). In many cases, the trienoic PG ($PGF_3\alpha$) have lower biological activity than the corresponding dienoic PG ($PGF_2\alpha$), which would contribute to improve fertility (Ambrose *et al*., 2002), as well as embryo survival. Ambrose *et al.* (2006) also observed lower pregnancy losses in cows fed flaxseed (9.8%) compared with those fed sunflower (27.3%). Moreover, the benefit of omega-3 fatty acids contained in flaxseed have resulted due to its supplementary effect that could have reduced the sensitivity of the corpus luteum to $PGF_2\alpha$. In agreement to the current study, Thatcher *et al.* (1986) 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) has also indicated that changes in luteolysis during the period of maternal recognition of pregnancy could also contribute to increase embryonic survival beyond day 28.

Conclusion

Results clearly indicate that Omega-3 rich feed (flaxseed) supplemented transition crossbred dairy cows appear to experience lowered PGFM but increased IGF-1 and progesterone concentrations than non-supplemented counterparts. Further, flaxseed supplemented cows tend to have earlier uterine involution, return to oestrus and breeding, improved conception rates as well as reduced pregnancy losses. Hence, supplementation of Transition period diets with PUFA/ Omega-3 fatty acid rich source feeds could have the potential to alleviate negative energy balance and related negative impacts on postpartum reproductive efficiency. More robust studies are needed to validate and expand on current promising results.

Acknowledgments

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Ethical Clearance

The entire ethical requirement in conducting a research process was followed and approval from the Ethics Committee was obtained for the adherence to all regulatory requirements and applicable guidelines.

Conflict of Interests

The authors declare that they have no competing interests.

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