

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/275052426>

Modulation of post-partum reproductive performance in dairy cows through supplementation of long- or short-chain fatty acids during transition period

Article in *J Anim Physiol a Anim Nutr* · April 2015

DOI: 10.1111/jpn.12304 · Source: PubMed

CITATIONS

14

READS

283

8 authors, including:



Girgo Galmessa Ulfina

Ethiopian Institute of Agricultural Research

65 PUBLICATIONS 172 CITATIONS

[SEE PROFILE](#)



Shiv Prasad Kimothi

Indian Council of Agricultural Research

23 PUBLICATIONS 115 CITATIONS

[SEE PROFILE](#)



Parvinder Singh Oberoi

National Dairy Research Institute

21 PUBLICATIONS 64 CITATIONS

[SEE PROFILE](#)



Rubina Kumari Baithalu

National Dairy Research Institute

55 PUBLICATIONS 259 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Study of critical control points for semen processing in dairy bovines [View project](#)



assesment of early pregnancy by analysisi of neutrophils dynamics & gene expression in dairy cattle [View project](#)

ORIGINAL ARTICLE

Modulation of post-partum reproductive performance in dairy cows through supplementation of long- or short-chain fatty acids during transition period

G. G. Ulfina, S. P. Kimothi, P. S. Oberoi, R. K. Baithalu, A. Kumaresan, T. K. Mohanty, P. Imtiwati and A. K. Dang

National Dairy Research Institute (NDRI) Karnal, Haryana, India

Summary

Thirty-six cross-bred cows were used to study the effect of long-chain (flaxseed) or short-chain (butyric acid) fatty acid supplementation on metabolic status, ovarian function and reproduction performance during transition period. Control cows received a routine feed of transition diet, while the cows in two treatment groups were supplemented with either 750-g crushed flaxseed or 250 g butyric acid per cow per day. Ovarian activity was monitored by transrectal ultrasonography on 10th, 20th and 30th days post-partum. Blood samples were collected by jugular venipuncture into heparinized polystyrene tubes; plasma was prepared and stored under -20°C until analysis. Results indicated that cows in flaxseed group were in positive energy balance as indicated by lower NEFA and Beta hydroxy Butyrate and higher glucose concentrations. Uterine involution was completed well within 30 days post-partum in all the cows in flaxseed fed group compared to 76.9% in butyric acid supplemented and 61.5% in control groups. The size of dominant follicle and corpus luteum was significantly higher ($p < 0.05$) for flaxseed group compared to control group, which in turn resulted in higher concentrations of plasma progesterone. Cows fed on diets supplemented with flaxseed exhibited post-partum heat earlier and bred sooner ($p < 0.05$) than control cows. It has been noticed that supplementation of flaxseed and butyric acid enhanced involution of uterus, early resumption of cyclicity and thereby early breeding. However, in view of the encouraging results obtained for flaxseed supplemented group, its organic nature and easier availability at farmer's gate, we concluded that flaxseed can be safely included in transition diet to modulate reproductive performance of dairy cattle.

Keywords involution, reproductive performance, flaxseed, butyric acid, transition period

Correspondence G. G. Ulfina, National Dairy Research Institute (NDRI), Karnal, Haryana 132001, India. Tel: +251-11-2363159; Fax: +251-11-2363137; E-mail: r.ulfinaa@yahoo.com

Received: 19 June 2014; accepted: 3 February 2015

Introduction

Lowered reproductive performance concomitant to steadily increasing milk production is a main concern for dairy industry world over. Numerous studies have reported that reproductive performance is compromised, primarily through delayed ovarian activity and reduced conception rates, by the demands of high milk yield (Nebel and McGilliard, 1993; Beam and Bulter, 1999). Although reduced reproductive performance has been associated with high milk yields (Dobson et al., 2009), measures of post-partum ovarian activity have been more closely related to energy balance. Transition period is one of the most challenging periods of the production cycle as it entails profound physiologic and metabolic changes that often

disrupt the homeostatic mechanisms of the cow. It coincides with high nutrient requirement for calf growth and milk synthesis and secretion while reduced in dry matter (DM) intake. This leads to negative energy balance in early lactation (Bell, 1995; Grummer, 1995). Defrain et al. (2005) viewed the 3 weeks before and 3 weeks after calving is the most important 6 weeks of the production cycle as it imparts the level of success realized during the subsequent lactation.

Reduced reproductive performance due to cross-breeding has direct and severe economic implications in terms of losses due to reduced production and additional cost on management (Mulligan et al., 2006). Although introduction of cross-breeding has certainly improved the milk production in the tropics, the

performance of cross-bred cows is affected by high temperature, poor feeding and susceptibility to diseases (Rao et al., 1995) making them a liability for the resource-poor rural farmers. Improved reproductive performance and reduced incidence of reproductive disorders have been observed by feeding supplemental fat to dairy cows (Grummer, 1995). Fats have been supplemented in ruminant ration to increase energy density, reduce dustiness in feed and aid in processing (Byers and Schelling, 1988). Because of the fact that it is rich in fat content, flaxseed is recommended to be used as fat source in the ration of dairy cattle. Petit et al. (2001) was the first to demonstrate that feeding flaxseed to dairy animals not only supports high level of milk production but also increases reproduction function as it is a rich source (58%) of omega-3 fatty acids.

Feeding flaxseed reduces the level of embryo deaths, increases follicle size, increases the size of corpus luteum (CL) and, therefore, increases reproductive performances in ruminants. This could be due to specific effect of fat on the pituitary gland, ovaries and uterus, mediated by the fatty acid composition of the fat sources (Hutchinson et al., 2012). Decrease in endometrial synthesis of $\text{PGF}_{2\alpha}$ through dietary supplementation of flaxseed (omega-3 fatty acids), viz. α -linolenic acid (Ambrose et al., 2006), has been shown to improve reproductive performance of dairy cows. The meagre information available on the significance of short-chain fatty acid, especially butyrate, in ruminants showed that Short Chain Fatty Acid are modulators of immunity and are an incredible source of energy (Lingaas and Tveit, 1992; Jianping, 2013). However, there is scanty information on the effect of short- and long-chain fatty acids as modulators of reproductive performances in Karan Fries (KF) cross-bred cows during transition period. Hence, the current study was designed to study the effect of both short-chain (butyric acid) and long-chain fatty acids (through flaxseed which is the main source of omega-3 fatty acid) on post-partum reproductive performance, uterine function and reproductive disorders in KF cows.

Materials and methods

Description of the study area

This study was conducted at Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal, India. The NDRI, Karnal, is located on 29°43'N latitude and 76°58'E longitudes at an altitude of 245 m 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 °C in May/June months of summer. The average annual rainfall is 700 mm, most of which is received from July to September.

Animals and feeding management

Karan Fries cross-bred cows (Tharparkar × Holstein Friesian) kept at Livestock Research Centre of the Institute were utilized for the study. Thirty-six multiparous pregnant cows entering their 2nd parity and above were selected, for 6 weeks (3 weeks before and after parturition) of the feeding period. The selected cows were randomly allotted on the basis of their parity and weight to one control and two treatments of 12 cows each under randomized complete block design (RCBD). Control cows (treatment 1) fed standard ration of the institute for pregnant and lactating cows. Under treatment 2, cows were fed control ration plus crushed flaxseed (omega-3) 750 g/day/head, and cows under treatment 3 fed control ration plus 250 g/day/head butyric acid over and above the routine feeding of the institute (Table 1). All cows were fed on isoenergetic diet formulated to meet or exceed the predicted requirements of National Research Council (NRC, 2001) and offered fresh chaffed green fodder *ad libitum* provided in three to four meals throughout the day. The concentrate was fed at the rate of 2.0 kg per animal for body maintenance. Milking cows were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield. Water was available *ad lib*.

Hormone and metabolite analyses

Blood samples were collected by jugular venipuncture on the days of -21, -14, -7, -2, +2, +7, +14, +21 before and after calving for estimation of hormones and metabolites. Immediately after the collection of blood, each blood sample was centrifuged at 4 °C at the rate of 3000 rpm for 20 min to separate the plasma from blood cells. The separated plasma samples were stored in cryovials at -20 °C till the assay for the hormone or estimation of metabolites was conducted.

The $\text{PGF}_{2\alpha}$ concentrations were measured as the venous concentration of 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$ (PGFM) using the B-Bridge 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$ (PGFM) immunoassay kit (catalogue no K3022-1 and K3022-5; B-Bridge international, Cuper-

Table 1 Proximate composition and fibre fractions of feed on DM basis along with Ca and P

Feedstuffs	DM%	CP%	EE	NDF	ADF	CF	TA	Ca	P
Maize (<i>Zea mays</i>)	17.31	8.82	2.52	57.22	37.64	28.8	9.43	0.18	0.15
Oats (<i>Avena sativa</i>)	15.14	10.26	1.92	47.89	29.65	26.29	9.53	0.45	0.34
Jowar (<i>Sorghum bicolor</i>)	23.00	7.64	1.76	27.81	38.42	31.42	9.25	0.39	0.19
Berseem (<i>Trifolium alexandrinum</i>)	14.92	14.6	2.98	47	38.9	26.96	12.7	2.32	0.4
Wheat straw	90.00	3.54	0.87	87.01	50.20	52	10.8	0.34	0.19
Concentrate	90.00	22.01	5.33	30.82	16.10	14.63	7.9	2.81	0.63
Flaxseed	92.01	18.34	39.88	27.98	21.94	6.88	3.04	2.3	6.22

DM, dry matter; CP, crude protein; EE, ether extract; NDF, neutral detergent fibre; ADF, acid detergent fibre; CF, crude fibre; TA, total ash; Ca, calcium; P, phosphorus.

tino, CA, USA). The progesterone concentrations were estimated using bovine progesterone hormone (P₄) ELISA test Kit (Endocrine Technologies, Newark, CA, USA). Glucose concentrations were estimated in plasma samples using B-Bridge International. Plasma Beta hydroxy Butyrate (BHBA) was estimated by 'β-Hydroxy butyrate (Ketone Body) ELISA Kit' of Qayee Bio-Technology Co., Ltd. Shanghai, China. Plasma non-esterified fatty acid (NEFA) was estimated by bovine NEFA kit; Qayee-Bio for Life Science, USA. All the estimations were carried out as per the procedure given by the manufacturers.

Reproductive management and disorders

The incidence of various reproductive disorders in treatment and control animals was recorded. A cow that had not expelled the foetal membranes within 12 h after calving (Roberts, 1971) was considered as a case of retention of foetal membranes (RFM). Cows those were not systemically ill, but had an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina, within 21 days post-partum, were classified as having clinical metritis (Sheldon et al., 2006). Clinical endometritis was characterized by the presence of purulent (>50% pus) uterine discharge detectable in vagina 21 days or more after parturition or mucopurulent discharge detectable in the vagina after 26 days post-partum (Sheldon et al., 2006). A cow was considered to be suffering from pyometra when there was an accumulation of purulent material within the uterine lumen in the presence of CL (Sheldon et al., 2006). The presence of a CL was determined through ultrasonographic examination and also on the basis of plasma progesterone profile of the cow.

Rectal palpation and ultrasonography

For confirming cervical and uterine involution, the organs were palpated per rectum on 10th, 20th and

30th days post-partum. The diameters of the organs were measured by subjectively estimating the thickness using the fingers to the nearest half-centimetre (Sheldon and Noakes, 1998). Complete involution was declared when the diameter of the erstwhile gravid uterine horn and cervix had reduced to the least possible values. Transrectal ultrasonography was used to determine the size of CL and dominant follicle. The number of class 1 (<6 mm), class 2 (6–9 mm) and class 3 (>9 mm) follicles located in each ovary was also recorded.

Heat detection and insemination

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

Statistical analysis

All data were subjected to ANOVA for RCBD using the procedure general linear model (GLM) of SAS (SAS Institute, 2002). The model used was as follows:

$$Y_{ijkl} = \mu + T_i + P_j + (T * P)_k + e_{ijkl}$$

where y_{ijkl} = mean of individual observation, T_i = the effect of treatment, P_j = effect of transition period (–21 to +21 related to calving, $(T * P)_k$ = interaction between treatment effect and the transition period, if any and e_{ijkl} = random error effect.

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

Body weight, body condition score (BCS) and dry matter intake (DMI)

The results of body weight, BCS and DMI have been presented in Table 2. Body weight and BCS increased before parturition and decreased after parturition and were found to be similar between diets. There was no significant difference among treatments for DMI, expressed in kilograms per day. However, DMI was tended ($p < 0.08$) to be lower for cows on butyric acid compared with those on either flaxseed or control diet. It was noticed that inclusion of crushed flaxseed at approximately 6% on DM basis had no negative effect on intake in the present study. Similarly, inclusion of rolled flaxseed at 9% on a DM basis (Ambrose *et al.*, 2006) and whole flax seed at level of 5–15% (Kennelly and Khorasani, 1993) or feeding formaldehyde-treated flaxseed at up to 17% of DM (Petit *et al.*, 2001) did not reduce DMI in dairy cows. The present result confirmed the earlier reports that untreated whole flaxseed (Sechiari *et al.*, 2003; Cortes *et al.*, 2010) and crushed flaxseed (Vikranjeet, 2013) had no effect on feed intake of cows in the mid- and early stages of lactation (Petit, 2002). DMI was slightly reduced in butyric acid supplemented cows which is in agreement with the observation of Senel and Own (1976) who found reduced feed intake by inclusion of 2% butyrate to hay concentrate ration of ruminants. But inclusion of 1% butyrate to hay concentrate ration did not reduce feed intake. In contrary to our results, Lingaas and Tveit (1992) reported that 200 g/day butyrate in feed did not influence feed intake.

Retention of foetal membranes and time required for its expulsion

Of 12 cows included in the control group, two cows had RFM. However, none of the cows in either omega-3 (flaxseed) or butyric acid supplemented groups had RFM. Cows fed on diets supplemented with flaxseed (750 g/day/h) took significantly ($p < 0.05$) lesser time to expel the placenta than cows kept in the control group (Table 2). The mean time required to expel the placenta in cows supplemented butyric acid was in between, and statistically similar to, the other two groups. This result showed the importance of flaxseed feeding during transition period as it helps to overcome the parturition stress and for expulsion of foetal membrane as early as possible which otherwise resulted in the RFM and subsequent reproductive consequences. The less time taken for expulsion of foetal membranes by the cows in ($p > 0.05$) butyric acid group compared to that of control ones also hinted the advantage of butyrate as transition feed, although caution should be taken in interpretation.

It is imperative to note that omega-3 source flaxseed reduced the time required for expulsion of foetal membrane through the anti-inflammatory action and enhancement of process of immunity which was later proved from the cows included in this group which showed complete involution and started cycling earlier than the control cows. Placental retention after normal parturition was more common when there was MHC class 1 compatibility between the dam and the calf, which implies a genetic similarity in the MHC

Table 2 DMI, body weight changes, calf birthweight and time required for expulsion of placenta in KF cows supplemented with omega-3 fatty acid (flaxseed) or butyric acid during transition period

Parameters	Treatments				p-Value
	FLAX	BUTY	CONT	Overall mean	
Initial body weight (kg)	449.5 ± 19.09	449.2 ± 13.78	449.4 ± 13.33	449.4 ± 14.33	0.999
Body weight (kg)					
Pre-partum	489.46 ± 20.088	492.76 ± 20.132	469.23 ± 12.552	483.82 ± 18.436	0.6082
Post-partum	431.38 ± 17.069	433.00 ± 16.354	428.61 ± 15.081	430.996 ± 16.361	0.9814
Mean difference	52.92 ± 4.757	61.84 ± 6.596	41.07 ± 6.176	51.94 ± 5.757	0.0561
DMI (kg/day)					
Pre-partum	10.92 ± 0.23	9.11 ± 0.45	9.68 ± 0.13	9.70 ± 0.12	0.2021
Post-partum	12.71 ± 0.11	11.21 ± 0.35	12.69 ± 0.08	12.00 ± 0.09	0.0811
Body condition score					
Pre-partum BCS	3.69 ± 0.108	3.51 ± 0.161	3.51 ± 0.133	3.53 ± 0.078 ^a	ns
Post-partum BCS	3.27 ± 0.161	3.21 ± 0.154	3.08 ± 0.092	3.19 ± 0.079 ^b	ns
Time required for expulsion of placenta (h)	4.29 ± 0.58 ^b	6.05 ± 0.75 ^{ab}	7.38 ± 1.08 ^a	5.90 ± 0.35	0.0426

FLAX, flaxseed; BUTY, butyric acid; CONT, control; KF, Karan Fries; BCS, body condition score; DMI, dry matter intake.

Means bearing different superscripts in a row differ significantly at $p < 0.05$.

locus between foetus and dam resulting in deficient alloreactivity of the maternal immune system against foetal antigens (Joosten and Hensen, 1992). Subsequently, this leads to a lack of cytokine production such as interleukin-2 and tumour necrosis factor alpha, necessary for the maturation and eventual shedding of the placenta (Mallick and Prakash, 2011). The role of immune suppression in RFM, as it relates to leucocyte activity, antioxidant capacity and steroid synthesis, is not completely understood (Gunnink, 1984; Peter and Bosu, 1987). So the maintenance of pregnancy requires suppression of the immune response to avoid rejection of the foetal-placental unit, and RFM might result from a failure to switch off these immune protective mechanisms, either because of immunosuppression or an interruption of the normal pre-partum hormonal changes. Kimura *et al.* (2002) observed that cows with RFM after normal parturition had decreased leucocyte chemotaxis and phagocytic activity before parturition. From the present results, it is suggested that a malfunction of immune system like RFM can be improved by supplementation of either flaxseed or butyric acid as they are known to have immunomodulatory effect.

Incidence of metritis, endometritis and pyometra

The post-partum reproductive abnormalities and involution status of control and experimental cows have been presented in Table 3. In the present study, we observed eight cases of metritis, nine cases of endometritis and five cases of pyometra (Table 3). Higher incidences of reproductive disorders were recorded in control and butyrate groups of cows than flaxseed supplemented ones. This result revealed the importance of flaxseed supplementation in improving the reproductive performance of dairy cows possibly

by enhancing the immunity and hence affording greater protection against harmful pathogens which are responsible for the uterine infections. It is also likely that the higher incidence of reproductive disorders (endometritis and pyometra) in the non-supplemented cows was due to a higher incidence of RFM in these cows. Additionally, the high incidence of reproductive disorders could have also contributed to delayed uterine involution and cyclicity in these cows. Mallick and Prakash (2011) also found high incidence of reproductive disorders in RFM cows. In most cows, the contamination is eliminated during the process of involution, together with extensive tissue remodeling. In 10–20% of cows, however, the infection is not controlled which may lead to chronic uterine inflammation (LeBlanc *et al.*, 2002; Sheldon *et al.*, 2006). Metritis, clinical endometritis, pyometra and subclinical endometritis are the most common forms of genital diseases in dairy cows, which may delay the complete regeneration of endometrium and disrupt the resumption of cyclic ovarian function resulting in postponement of the first insemination (AI), increasing the service of AIs per conception, and thus prolonging the calving interval and decreasing the calving rate (Hussain and Daniel, 1991a; Sheldon *et al.*, 2006). Foldi *et al.* (2006) reported that the majority (80–90%) of bovine uteri become contaminated with bacteria after calving. Endometritis caused by uterine infection after calving reduces fertility and causes major economic losses to the dairy industry (Theerawat *et al.*, 2012). The effect of these disorders on the subsequent reproductive performance also clearly showed the trend towards an improvement in flaxseed supplemented cows in all the reproductive parameters considered. It is also learnt that these periparturient and/or early post-partum disorders hinder the performance of dairy cows even though modulation of these problems by flaxseed seemed to be encouraging.

Uterine involution

There was an early uterine involution ($p < 0.01$) in cows under feeding treatment compared to the control cows (Table 3). The observation that cross-bred supplemented cows exhibited early uterine involution in comparison to control cows also indicates that there is better immunity in the supplemented cows which ensures greater protection against infections and hence faster recovery and early involution. Results indicated that 100% of the cows from flaxseed supplemented group completed uterine involution before 30 days against 76.9% for the butyric acid and 61.5%

Table 3 Post-partum reproductive abnormalities and Involution status in KF cows supplemented with omega-3 fatty acid (flaxseed) or butyric acid during transition period

Disorders	Treatment		
	FLAX	BUTY	CONT
RFM	0	0	2
Metritis	2	2	4
Endometritis	2	2	5
Pyometra	1	1	3
Dystocia	0	1	2
Involution (%) at 30 day	100	76.9	61.5

FLAX, flaxseed; BUTY, butyric acid; CONT, control; KF, Karan Fries cows; RFM, retention of foetal membranes.

for the non-supplemented cows. This is may be because of the anti-inflammatory and enhancement of immune process of omega-3 FA source of flaxseed. This was further proved by enhanced days to first service (AI) (Table 4) which is significantly shorter ($p < 0.05$) for flaxseed supplemented cows than the control cows. Beyond its anti-inflammatory property, omega-3 FA source flaxseed has a potent advantage of enhancing post-partum performance by attenuation of PGF_{2α} and thereby increasing CL size for increased progesterone production (Table 4). α-Linolenic acid (ALA), an omega-3 fatty acid, present in flaxseed suppresses PGF_{2α} synthesis by inhibiting the endometrial expression of COX-2, a rate limiting enzyme for PGF_{2α} (Palin et al., 2005). Moreover, ALA inhibits PGF_{2α} release through decreased availability of arachidonic acid and through increased competition of ALA with arachidonic acid for binding to COX-2 (Mattos et al., 2002). In addition, analysis for metabolite markers also indicated higher glucose ($p < 0.05$), lower BHBA ($p < 0.01$) and NEFA ($p < 0.001$) in omega-3 source flaxseed supplemented cows as compared to control

ones, indicating that these animals are in a better energy balance than the other groups (Table 5).

The low concentrations of plasma PGFM (pg/l) following flaxseed supplementation as compared to control cows indicated attenuation in PGF_{2α} release in dairy cows (Table 5). Uterine flushing of non-inseminated dairy cattle revealed lower PGFM concentrations subsequent to whole flaxseed supplementation (9.1% DM) for 4 weeks (Petit et al., 2007). Supplementation of post-partum dairy buffalo with fish meal, another source of omega-3 fatty acids, for 30 days also decreased plasma PGFM (Malik et al., 2011a). Nazir et al. (2012) also found lower PGFM concentration, higher size of CL and higher production of progesterone production in crushed flaxseed supplemented buffalo in comparison to non-supplemented buffalo.

Follicular development and size of corpus luteum

The dominant follicle and CL size recorded for flaxseed supplemented cows were significantly larger

Table 4 Follicular size, classes of follicles and CL size, DFH and DFS in KF cows supplemented with omega-3 fatty acid (flaxseed) or butyric acid during transition period

Item	Treatment			p-Value
	FLAX	BUTY	CONT	
Dominant follicle diameter, mm	12.78 ± 0.62 ^a	10.54 ± 0.57 ^b	9.34 ± 0.60 ^b	0.0017
CL diameter, mm	18.40 ± 0.29 ^a	13.72 ± 1.44 ^b	11.44 ± 0.97 ^b	0.0008
Classes of follicles, no				
Small, 3–4.9 mm	3.5 ± 0.4 ^a	2.8 ± 0.3 ^{ab}	2.3 ± 0.3 ^{bc}	<0.05
Medium, 5–9.9 mm	2.5 ± 0.6 ^a	1.0 ± 0.2 ^b	1.5 ± 0.3 ^a	<0.05
Large ≥10.0 mm	1.1 ± 0.1	1.2 ± 0.2	1.0 ± 0.1	>0.05
Days to first heat (DFH), day	43.62 ± 3.85 ^b	59.58 ± 6.06 ^{ab}	76.83 ± 10.08 ^a	0.0196
Days to first service (DFS), day	72.25 ± 6.23 ^b	85.11 ± 10.20 ^{ab}	116.0 ± 10.20 ^a	0.0241

FLAX, flaxseed; BUTY, butyric acid; CONT, control; DFH, day to first heat; DFS, day to first service; CL, corpus luteum; KF, Karan Fries. Means bearing different superscripts in a row differ significantly at $p < 0.05$.

Table 5 Overall mean of blood metabolites and hormones in KF cows fed on diets supplemented with omega-3 fatty acid or butyric acid during transition period

Plasma hormones/metabolites	Treatment			p-Value treatment	p-Value TP
	FLAX	BUTY	CONT		
PGFM, pg/l	460.1 ± 66.69 ^b	720.0 ± 15.37 ^a	836.5 ± 82.63 ^a	0.0020	0.0178
Progesterone, ng/ml	3.18 ± 0.179 ^a	2.38 ± 0.341 ^{ab}	2.02 ± 0.252 ^b	0.0263	0.0022
Glucose, mg/dl	59.95 ± 0.599 ^a	59.36 ± 0.873 ^a	56.84 ± 1.433 ^b	0.0178	0.4230
BHBA, mmol/l	0.611 ± 0.023 ^b	0.826 ± 0.041 ^a	0.865 ± 0.059 ^a	0.0014	0.0546
NEFA, mmol/l	0.263 ± 0.075 ^a	0.6097 ± 0.162 ^b	0.741 ± 0.219 ^b	0.0001	0.5250

FLAX, flaxseed; BUTY, butyric acid; CONT, control; KF, Karan Fries; PGFM, 13,14-dihydro-15-keto PGF_{2α}. Means bearing different superscripts in a row differ significantly at $p < 0.05$.

($p < 0.01$) than the two other groups (Table 4). However, the larger size of dominant follicle and CL recorded in butyric acid supplemented cows were non-significant ($p > 0.05$) as compared to control cows. Similarly, Staples et al. (2000) and Ambrose et al. (2006) reported significantly larger ovulatory follicles in cows of the flaxseed group compared with those in the control (sunflower) group. The total number of follicles also tended to be ($p = 0.08$) higher for flaxseed group as compared to the other groups. Although there was no difference in the mean number of follicle classes, Petit et al. (2001) and Petit and Twagrumungu (2006) observed a higher ($p = 0.09$) number of follicles for cows fed Megalac than for those fed flaxseed. It has been observed that the larger mean diameter of dominant follicle and CL in flaxseed supplemented cows was resulted in significantly higher production of progesterone ($p < 0.05$) (Table 4), as opposed to Ambrose et al. (2006) who reported plasma progesterone concentrations remained unaffected after supplementation. Staples et al. (2000) suggested that polyunsaturated fatty acids altered ovarian and uterine dynamics as a result of greater induction of mRNAs for PG endoperoxide synthase-2 from uterine biopsy for cows fed Linoleic acid.

Post-partum oestrus and days to first service

The mean number of days taken to express first heat (DFH) for cows fed on diets supplemented with flaxseed was significantly lesser ($p = 0.0196$) than control cows (Table 4). The mean value of DFH for cows fed on diets supplemented with butyric acid was in between flaxseed and control cows, and statistically similar to both groups. This result clearly indicated that omega-3 source flaxseed improves oestrus detection and thereby reproductive performances of dairy cows. Low level of plasma $\text{PGF}_{2\alpha}$ and high progesterone level (Table 5) at early post-partum in flaxseed supplemented cows also substantiates the significance of flaxseed in improving early oestrus detection in dairy animal management. In contrary, Burke et al. (1997) reported that feeding fish meal (another source of omega-3) had no effect on oestrous detection of early lactating dairy cows prior to oestrus synchronization. The mean DFH for flaxseed group in present study was also shorter than the mean DFH observed for in the same breed (Biplob, 2011), indicating the animals of the present study were in better management condition. It is well noted that the resumption of oestrus cycle after parturition depends on the nutritional status, body energy reserves and blood glucose

level of the animal. Even though blood glucose is the main source of energy for function (Rabiee et al., 1997) which can influence bovine thecal cell steroidogenesis *in vitro* (Stewart et al., 1995), the type or sources of the energy seem to be the main factor in the present study as all groups were provided isoenergetic diets.

Table 5 presents the least square means of days to first service in KF cows fed on diets supplemented with flaxseed or butyric acid during transition period. Significantly ($p < 0.05$) lesser days to first A.I. was recorded for cows fed on diets supplemented with flaxseed compared with cows fed on control diets (Table 4). The value recorded in the present study for both treatment groups was also lesser than that reported by Biplob (2011), for the same breed of cattle. Susavi (2013) also recorded a longer days to first service for RFM- than for non-RFM-affected KF cows. The high performance of flaxseed group of cow in this respect can be explained to the anti-inflammatory and enhancement of immune process of flaxseed which could help early involution of reproductive organ and resumption of oestrus as opposed to control cows. The cows supplemented with butyric acid also performed better than that of control cows although the mean difference was not significant. To the best of our knowledge, this is the first study which shows beneficial effects of flaxseed on early commencement of uterine involution, cyclicity and early insemination which are important parameters for fertility enhancement. This may be due to the better immunity enhancement of flaxseed supplementation which is the key to early uterine involution and cyclicity commencement in these animals.

Conclusions

Results clearly indicate that supplementation of omega-3 fatty acid or butyric acid resulted in healthier cows that expelled the placenta sooner, had earlier uterine involution and required lesser days to first heat and bred earlier as compared to non-supplemented cows. Results from metabolic markers also clearly indicated that cows in flaxseed group had a better energy balance than the other groups. Therefore, it can be concluded that omega-3 fatty acid (crushed flaxseed) or butyric acid supplementation during transition period improves the reproductive performances of KF cows. Further, keeping in view the encouraging results obtained in flaxseed supplemented cows, its organic nature and easier availability at farmer's gate, flaxseed can be recommended for

inclusion in transition diet to modulate reproductive performance of dairy cattle.

Acknowledgements

The authors are highly thankful to the Indo-African fellowship programme and the Director of National Dairy Research Institute (NDRI) for providing finan-

cial support and necessary facilities, respectively, for carrying out the research work. The authors also wish to thank Dr. Muzammil and Dr. Shilpi, the Ph.D. scholars of the LPM department for their help during measurement of reproductive organs using ultrasonography and Dr. Mandheer during analysis of hormones and metabolites.

References

- Ambrose, J. D.; Kastelic, J. P.; Corbett, R.; Pitney, P. A.; Petit, H. V.; Small, J. A.; Zalkovic, P., 2006: Lower pregnancy losses in lactating dairy cows fed a diet enriched in α -linolenic acid. *Journal of Dairy Science* **89**, 3066–3074.
- Beam, S. W.; Bulter, W. R., 1999: Effects of energy balance on follicular development and first ovulation in postpartum dairy cows. *Journal of Reproduction and Fertility. Supplement* **54**, 411–424.
- Bell, A. W., 1995: Regulation of organic nutrient metabolism during transition from late pregnancy to early lactation. *Journal of Animal Science* **56**, 2804–2818.
- Biplob, K. R., 2011: Body condition score in relation to certain physiological and performance parameters of Sahiwal and Karan Fries cows. PhD thesis, National Dairy Research Institute, NDRI, Karnal, Haryana, India.
- Burke, J. M.; Staples, C. R.; Risco, C. A.; De la Sota, R. L.; Thatcher, W. W., 1997: Effect of ruminant grade menhaden fish meal on reproductive performance of lactating cows. *Journal of Dairy Science* **80**, 3386–3398.
- Byers, F. M.; Schelling, G. T., 1988: Lipids in ruminant nutrition. In: (D. C. Church, ed.) *The Ruminant Animal Digestive Physiology and Nutrition*. Prentice Hall, Englewood Cliffs, NJ, pp. 298–312.
- Cortes, C.; da Silva-Kazama, D. C.; Kazama, R.; Gagnon, R.; Bencheur, C.; Santos, G. T. D.; Zeoula, L. M.; Petit, H. V., 2010: Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. *Journal of Dairy Science* **93**, 3146–3157.
- Defrain, J. M.; Hippen, A. R.; Kalscheur, K. F.; Patton, R. S., 2005: Effect of feeding propionate and calcium salts of long-chain fatty acids on transition dairy cow performance. *Journal of Dairy Science* **88**, 983–993.
- Dobson, H.; Smith, R. F.; Royal, M. D.; Knight, C. H.; Sheldon, C. I. M., 2009: The high producing dairy cow and its reproductive performance. *Reproduction in Domestic Animals* **42**(Suppl 2), 17–23.
- Foldi, J.; Kulcsar, M.; Pecs, A.; Huyghe, B.; de Sa, C.; Lohuis, J. A.; Cox, P.; Huszenicza, G., 2006: Bacterial complications of postpartum uterine involution in cattle. *Animal Reproduction Science* **96**, 265–281.
- Grummer, R. R., 1995: Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of Dairy Science* **73**, 2820–2833.
- Gunnink, J. W., 1984: Prepartum leukocytic activity and retained placenta. *Veterinary Quarterly* **6**, 52–54.
- Hussain, A. M.; Daniel, R. C. W., 1991a: Bovine normal and abnormal reproductive and endocrine functions in the postpartum period: a review. *Reproduction in Domestic Animals* **26**, 101–111.
- Hutchinson, I. A.; Hennessy, A. A.; Waters, S. M.; Dewhurst, R. J.; Evans, A. C. O.; Ioneran, P.; Butler, S. T., 2012: Effect of supplementation with different fat sources on the mechanisms involved in reproductive performance in lactating dairy cattle. *Theriogenology*, **78**, 12–27.
- Jianping, Y., 2013: Butyric acid in ghee heals the mind and body. <http://whole-healthsource.blogspot.com/2009/12/butyric-acid-ancient-controller-of.html>.
- Joosten, I.; Hensen, E. J., 1992: Retained placenta: an immunological approach. *Animal Reproduction Science* **28**, 451–461.
- Kennelly, J. J.; Khorasani, G. R., 1993: Influence of flaxseed feeding on fatty acid composition on cows milk. In: J. F. Carter (ed.) *Proceedings of 54th Flaxseed Inst. Conf.*, Fargo, ND. North Dakota State University, Fargo, ND.
- Kimura, K.; Goff, J. B.; Kehrl, M. E.; Reinhardt, T. A., 2002: Decreased neutrophil function as a cause of retained placenta in dairy cattle. *The Journal of Reproduction and Development* **85**, 544–550.
- LeBlanc, S. J.; Duffield, T. F.; Leslie, K. E.; Bateman, K. G.; Keefe, G. P.; Walton, J. S.; Johnson, W. H., 2002: Defining and diagnosing postpartum clinical endometritis and its impact on reproductive performance in dairy cows. *Journal of Dairy Science* **85**, 2223–2236.
- Lingaas, F.; Tveit, B., 1992: Etiology of acetoneuria in Norwegian cattle: effect of butyric acid, valeric acid & putrescine. *Journal of Dairy Science* **75**, 2433–2439.
- Malik, A. A.; Gandotra, V. K.; Brar, P. S.; Ghuman, S. P. S.; Dhaliwal, G. S., 2011a: Attenuation of luteolytic response following fish meal supplementation in dairy buffaloes. *Animal Reproduction Science* **126**, 45–49.
- Mallick, S.; Prakash, B. S., 2011: Effects of supplementation of *Tinospora Cordifolia* to crossbred cows peripartum. *Animal Reproduction Science* **123**, 5–13.
- Mattos, R.; Staples, C. R.; Williams, J.; Amorcho, A.; McGuire, M. A.; Thatcher, W. W., 2002: Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. *Journal of Dairy Science* **85**, 755–764.
- Mulligan, F. J.; O'Grady, L.; Rice, D. A.; Doherty, M. L., 2006: A herd health approach to dairy cow nutrition and production diseases of the transition cow. *Animal Reproduction Science* **96**, 331–353.
- National Research Council (NRC), 2001: *Nutrient Requirements of Dairy Cattle*, 7th rev. edn. Natl. Acad. Press, Washington, DC.
- Nazir, G.; Ghuman, S. P. S.; Singh, J.; Honparkhe, M.; Ahujab, C. S.; Dhaliwal, G. S.; Sangha, M. K.; Saijpaul, S.; Agarwal, S. K., 2012: Improvement of conception rate in postpartum flaxseed supplemented buffalo with Ovsynch + CIDR protocol. *Animal Reproduction Science* **137**, 15–22.
- Nebel, R. L.; McGilliard, M. L., 1993: Interactions of high milk yield and reproductive performance in dairy cows. *Journal of Dairy Science* **76**, 3257–3268.
- Palin, M. F.; Broch-Guadreau, K.; Beaudry, D.; Small, J.; Petit, H. V., 2005:

- Effects of feeding flaxseed on cyclooxygenase 2 (COX-2) and peroxisome proliferator-activated receptors (PPAR) delta and gamma mRNA levels at the time of maternal recognition of pregnancy in Holstein cows. *Biology of Reproduction* (Special Issue), 142.
- Peter, A. T.; Bosu, W. T. K., 1987: Periparturient endocrine changes associated with retained placenta in dairy cows. *Theriogenology* **28**, 383–394.
- Petit, H. V., 2002: Digestion, milk production, milk composition, and blood composition of dairy cows fed whole flaxseed. *Journal of Dairy Science* **85**, 1482–1490.
- Petit, H. V.; Twagiramungu, H., 2006: Conception rate and productive function of dairy cows fed different fat sources. *Theriogenology* **66**, 1316–1324.
- Petit, H. V.; Dewhurst, R. J.; Proulx, J. G.; Khalid, M.; Haresign, W.; Twagiramungu, H., 2001: Milk production, milk composition, and reproductive function of dairy cows fed different fats. *Canadian Journal of Animal Science* **81**, 263–271.
- Petit, H. V.; Palin, M. F.; Doepel, L., 2007: Hepatic lipid metabolism in transition dairy cows fed flaxseed. *Journal of Dairy Science* **90**, 4780–4792.
- Rabiee, A. R.; Lean, I. J.; Gooden, J. M.; Miller, B. J.; Scaramuzz, R. J., 1997: An evaluation of trans-ovarian uptake of metabolites using arterio-venous difference methods in dairy cattle. *Animal Reproduction Science* **72**, 49–58.
- Rao, S. V. N.; Venkatasubramanian, V.; Wit, J. D., 1995: Consequences of cross-breeding programme in India. *Economic and Political Weekly* **30**, A112–A116.
- Roberts, S. J., 1971: *Veterinary Obstetrics and Genital Diseases*. Ithaca, NY.
- SAS Institute, 2002: *SAS Software User's Guide. Version 9.3*. SAS Inst., Cary, NC.
- Sechiari, P.; Antogiovanni, M.; Mele, M.; Serra, A.; Buccioni, A.; Ferruzzi, G.; Paoletti, F.; Petacchi, F., 2003: Effect of kind of dietary fat on the quality of milk fat from Italian Friesian cows. *Livestock Production Science*, **83**, 43–52.
- Senel, S. H.; Own, F. G., 1976: Relation of dietary acetic and butyric acids to intake, digestibility, lactation performance, and ruminal and blood levels of certain metabolites. *Journal of Dairy Science* **50**, 327–333.
- Sheldon, I. M.; Noakes, D. E., 1998: Comparisons of three treatments for bovine endometritis. *The Veterinary Record* **142**, 575–579.
- Sheldon, I. M.; Lewis, G.; LeBlanc, S.; Gilbert, R., 2006: Defining postpartum uterine disease in dairy cattle. *Theriogenology* **65**, 1516–1530.
- Staples, C. R.; Wiltbank, M. C.; Grummer, R. R.; Guenther, J.; Sartori, R.; Diaz, F. J., 2000: Effect of long chain fatty acids on lactation performance and reproductive tissues Holstein cows. *Journal of Dairy Science* **83**(Suppl. 1), 278.
- Stewart, R. E.; Spicer, L. J.; Hamilton, T. D.; Keefer, B. E., 1995: Effects of insulin-like growth factor I and Insulin on proliferation and on basal and luteinizing hormone-induced steroidogenesis of bovine thecal cells: involvement of glucose and receptors for insulin-like growth factor I and luteinizing hormone. *Journal of Animal Science* **73**, 3719–3731.
- Susavi, K., 2013: Identification of risk factors and peri-partum metabolic indicators for retention of foetal membranes in bovines. MSc thesis, National Dairy research Institute, Karnal-Haryana, India.
- Theerawat, S.-U.; Lavender, C. R. M.; Cheng, Z.; Fouladi-Nashta, A. A.; Claire Wathes, D., 2012: Responses of the bovine endometrium to infection. BOR Papers in Press. Published on October 17, 2012 as DOI:10.1095/biolreprod.112.102376.
- Vikranjeet, 2013: Supplementation effect of flaxseed on production performance and nutrient utilization in lactating cows. MSc thesis, National Dairy Research Institute, Karnal.