



Effect of Bypass Fat Supplementation on Reproductive Advancement of Anoestrus Dairy Cows

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ABSTRACT

Background: Negative energy balance (NEBAL) during early lactation is one of the important causes for reproductive failure in lactating cows. Energy dense fats helps in improving the energy status during early lactation. Fish oil containing Omega-3 fatty acids support the corpus luteum and reduced the early embryonic death. Beneficial effects of feeding bypass fatty acids for amelioration of infertility associated with negative energy balance are not explored much among dairy cows in India.

Methods: To investigate the effects of palm and fish oil-based bypass fat supplementation, 24 anoestrus cows were divided into three groups namely, Control (AN-C), Bypass Fat of Palm Oil (AN-BPF-PO) and Bypass Fat of Fish Oil (AN- BPF-PO + BPF-FO). Cows in the control group were as fed as per farm practice, while AN-BPF-PO group were supplemented with palm oil-based bypass fat @ 100 g + 10 g/Kg milk and AN- BPF-PO + BPF-FO group were fed similar to AN-BPF-PO and additionally provided with fish oil-based bypass fat @ 300 g twice weekly over a period of 90 days. Onset of estrus, conception rate, changes in blood biochemical parameters were studied.

Result: Cows under AN- BPF-PO + BPF-FO group had significantly ($P < 0.05$) shorter days for onset of cyclicity when compared to AN-C and AN-BPF-PO group cows. Within the experimental period, 3(37.50%), 6(75.00%) and 6(75.00%) cows became pregnant with the overall conception rate of 27.27, 54.55 and 54.55 %, in the AN-C, AN-BPF-PO and AN- BPF-PO + BPF-FO groups. NEFA level in the AN-BPF-PO was significantly lower from day 42 to 77 compared to the control group. These findings suggest the potentially beneficial effect of palm and fish oil-based bypass fat supplementation on reproductive enhancement in anoestrus cows.

Key words: Anoestrus, Bypass fat, Dairy cow, Fish oil, Palm oil.

INTRODUCTION

Reproductive disorders occurring during early and mid-lactation significantly contribute to prolonged calving interval in dairy cows. Anoestrus is one of major forms of infertility and the incidences ranged from 7.72 to 60.00% among indigenous cows (Rahman *et al.*, 2024), 13.13 to 40.20% in crossbred cows and 20.00 to 67.00 % in buffaloes. During early lactation, the peak in feed intake lags behind the peak in milk production and this misalignment results in negative energy balance. Studies indicated that 20-50% of dairy cows and 18-32% buffaloes were affected by NEBAL and ketosis (Ghahfarokhi *et al.*, 2018). NEBAL prompts the mobilization and breakdown of adipose tissue and proteins, leading to ketone body formation, increase in non-esterified fatty acids (NEFA) and urea level in the body. These alterations disrupt the follicular environment, impair the oocyte quality and its developmental potential and decrease estradiol synthesis. Energy dense fats play crucial role in improving the energy status during early lactation, offering approximately 2.5-times the energy concentration of cereals and supplies energy. However, unprotected fats such as vegetable oils above 1% DMI level can cause changes in the microbial fermentation. Bypass fat is a potential energy supplement in dairy cow rations and had no adverse effects on rumen pH or fiber digestion (Palmquist and Jenkins, 2017). Omega-3 fatty acids have been reported to promote developmental competence of

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follicles; act as signaling molecules during embryo elongation (Ribeiro *et al.*, 2016), participate in membrane stabilization, gene expression, inter and intra cellular transport. Fish oil rich in Omega-3 fatty acids inhibits the action of cyclooxygenase-2 (COX2) on the corpus luteum, increased the lifespan and supporting constant production of the progesterone thereby reducing early embryonic death (Hansen *et al.*, 1998). The present experiment was designed to study the effects of bypass fat of palm and fish oils on the reproductive performance in anoestrus cows.

MATERIALS AND METHODS

Animal ethics

This experiment was a part of the research project Augmentation of fertility in Jersey crossbred cows through nutritional and hormonal interventions (Project No. B-57). The project was approved by the Institute Research Council of ICAR-National Dairy Research Institute, Karnal, India.

Animal selection

Experiment was carried out in Jersey crossbred cows maintained at ICAR-National Dairy Research Institute (NDRI), Eastern Regional Station, Kalyani, West Bengal, India. Based on gynaeco-clinical examination, 24 true anoestrus cows within 1 to 3 lactations were selected. Cows were divided into three groups namely, Control (AN-C), Bypass Fat of Palm Oil group (AN-BPF-PO) and Bypass Fat of Palm Oil and Fish Oil group (AN-BPF-PO + BPF-FO), consisting eight cows in each group.

Supplementing bypass fat

For the bypass fat supplementation, palm oil-based bypass fat was procured from ICAR-Central Coastal Agricultural Research Institute, Old Goa, Goa, India. The fish oil was procured from Avasta Pharma, Maharashtra, India. Fish oil-based bypass fat was prepared as per Naik, (2013). The control group cows feeding was done according to farm protocol following NRC, 2001. Ration of cows in the AN-BPF-PO group was similar to control group and additionally they were supplemented with palm oil-based bypass fat @ 100 g + 10 g/Kg milk produced for a period of 90 days. AN-BPF-PO + BPF-FO group ration was similar to AN-BPF-PO group and in addition, fish oil-based bypass fat was added @ 300 g twice weekly as a source of DHA and EPA for the same duration.

Collection of blood samples and biochemical analysis

Blood samples were collected at weekly intervals in EDTA tubes (10%), plasma was separated and stored at -20°C till analysis. Copper soap extraction method modified by Shipe *et al.* (1980) was adopted for the determination of plasma NEFA. Progesterone was estimated using ELISA Kit (G-Biosciences, St. Lois, MO, USA ITE050018) following suppliers guideline.

Reproductive parameters

Estrus was observed based on external manifestations, confirmed by rectal examination and intensity scored as per Dash, (1980). Ovarian activity was monitored at weekly intervals through trans-rectal ultrasound examination (DIGI 1100 CD-E VET, SSMED Ltd.) and follicles were classified on the basis of size *i.e.*, small 2 - 4.99 mm; medium 5-7.99 mm, large 8-11.99 mm and ovulatory follicles >12 mm. Days for the onset of estrus, conception rate, number of inseminations per conception in the dairy cows under treatment and control were recorded. Pregnancy diagnosis was carried out at 32 to 35 days post insemination by ultrasonography.

Statistical analysis

One way ANOVA, General linear model was followed for analysis of data recorded under various experiments in this study. The data were analysed with the help of IBM SPSS software 26.0 version. The post hoc comparison (Duncan's multiple range test) and considered a significantly different when $P < 0.05$.

RESULTS AND DISCUSSION

Changes in body weight (kg)

Body weight changes during 90th day supplementation period in anoestrus dairy cows was 334.44 ± 11.74 to 339.50 ± 12.19 kg in AN-C, 307.38 ± 13.88 to 311.88 ± 14.16 kg in AN-BPF-PO, 310.69 ± 18.00 to 316.25 ± 17.18 kg in AN-BPF-PO + BPF-FO group. There was no significant difference in body weight was observed between and within AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO groups respectively. Naik *et al.* (2009), Khalil *et al.* (2012) and Manriquez *et al.* (2019) reported higher BCS in the bypass fat supplemented group compared to control cows during early postpartum. But, similar to the current study, Arun (2022) did not find any significant changes in body weight (313.0 ± 4.05 vs 344.0 ± 2.84 kg) in the anoestrus cows of control and bypass fat supplemented groups. This might be due to the fact that the anoestrus cows were already in mid to late stage of their lactation and there might be less mobilization of body reserves when compared to early lactation period.

Reproductive parameters

No significant differences in duration of estrus were recorded between the cows under AN-C (10.94 ± 1.14 hrs), AN-BPF-PO (12.95 ± 1.59 hrs), AN-BPF-PO + BPF-FO (14.28 ± 0.95 hrs.), groups respectively, when they started to become cyclical. Intensity of estrus was categorized into faint, weak, expressive and strong. Faint estrus was detected only in one cow (12.50%) in the control group but was not observed in the bypass fat supplemented groups. 2 cows (25.00%) each in the AN-C and AN-BPF-PO group and 1 cow (12.50%) in AN-BPF-PO + BPF-FO group exhibited weak intensity of estrus. 4 cows (50.00%) each in AN-BPF-PO and AN-BPF-PO + BPF-FO group and 3 cows (37.50%) in the control group shown expressive intensity of estrus. Strong intensity of estrus was observed in 2 cows (25.00%) each in AN-C and AN-BPF-PO group and 3 cows (37.50%) in AN-BPF-PO + BPF-FO group, respectively. Arun, (2022) reported longer duration of estrus in cows supplemented with bypass fat, averaging 13.18 ± 0.45 hours, in contrast to the control group, which had a significantly ($P < 0.05$) shorter estrus duration of 9.33 ± 0.065 hours. Kalita *et al.* (2018) reported a longer estrus duration of 22.80 ± 0.62 hrs, ranging from 19 to 25 hrs, in anoestrus cows that were supplemented with minerals, bypass fat and hormonal treatment. Similarly, Pradhan (2021) found that the duration of estrus ranged from 24.25 to 24.50 hrs in anoestrus Binjharipuri cattle after supplementing fatty

acids along with mineral mixture. Dixit *et al.* (2023) reported estrus duration of 18.57 to 32.75 hrs in postpartum cows supplemented with mineral mixture and bypass fat. Multiple factors such as number of cows in estrus at a time, sexually active group, parity, BCS, production /unit of metabolic body weight, changes in body weight during the transition period are governing the estrus duration (Van Vliet and Van Eerdenburg, 1996). In the present study, anoestrus cows under AN-BPF-PO + BPF-FO had better expression of estrus compared to the control. Fatty acids of bypass fat improve cholesterol level in the circulation and improved the steroidogenesis leading to a better expression of estrus (Prajapati *et al.*, 2022).

Number follicles

The number of small follicles ranged from 1.67 ± 0.33 to 6.17 ± 0.48 , 2.00 ± 1.06 to 6.17 ± 1.54 and 1.5 ± 0.34 to 5.67 ± 0.80 and number of medium follicles ranged from 0.83 ± 0.31 to 1.83 ± 0.60 , 1.17 ± 0.40 to 4.00 ± 1.26 and 1.33 ± 0.21 to 2.50 ± 0.56 ; and the average number of larger follicles ranged from 0.17 ± 0.17 to 0.83 ± 0.65 , 0.17 ± 0.17 to 1.33 ± 0.56 and 0.17 ± 0.17 to 1.33 ± 0.33 in the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO groups, respectively. No significant differences ($P > 0.05$) were observed between the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO groups in the average number of small, medium and large follicles recorded for the entire period of supplementation. Bilby *et al.* (2006) found that dietary supplementation with calcium salts of fish oil fatty acids led to an increase in the number of follicles measuring 2.0-5.0 mm in dairy cows during early lactation. Similarly, Zachut *et al.* (2010) reported increase in the number of small follicles following supplementation of fatty acids. However, Heravi Moussavi *et al.* (2007) found that the addition of fish meal or calcium salts of fish oil fatty acids had no significant effect on either the number or size of follicles. Similarly, Childs *et al.* (2008) involving beef heifers after estrus synchronization, the addition of fish meal or calcium salts of fish oil fatty acids did not have a significant impact on the number or size of follicles. This might be due to differences in the fatty acid composition and energy sufficiency in the control and treatment group (Mattos *et al.*, 2000). Kinkar (2021) reported that in postpartum cows, there were 0.74 ± 0.09 and 0.91 ± 0.07 number of large-sized follicles in the control and bypass fat supplemented groups, respectively. In the current study, the average number of large follicles for the entire study period was 0.50 ± 0.10 , 0.61 ± 0.09 and 1.55 ± 0.31 in the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO groups, respectively. This suggests that EPA, DHA and alpha-linolenic acid (PUFA) had some impact on follicular growth. Prusty *et al.* (2023) also suggested that PUFA stimulate ovarian follicle growth, leading to an increase in both follicle number and size, ultimately improving reproductive performance in cattle.

7(87.50%), 8(100%) and 8(100%) number of cows under AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO group, came into estrus within 82.71 ± 2.63 , 72.75 ± 4.21 and

63.75 ± 5.95 days after the initiation of bypass fat supplementation, respectively. Cows under AN-BPF-PO + BPF-FO group had significantly ($P < 0.05$) shorter days for onset of cyclicity when compared to AN-C and AN-BPF-PO group cows. Further, within the experimental period of 90 days, 3 (37.50%), 6 (75.00%) and 6 (75.00%) cows became pregnant with the overall conception rate of 27.27, 54.55 and 54.55% and with number of services per conception of 3.33, 2.00 and 1.83 respectively, in the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO groups. Fish oils, which provide EPA, DHA and ALA, contribute by suppressing PG synthesis through the inhibition of the cyclooxygenase enzyme (Rahbar *et al.*, 2014). This inhibition of PGF 2α may lead to the persistence and growth of corpus luteum (CL), preventing its lysis. Indeed, the study conducted by Prusty *et al.* (2023) aligns with the notion that polyunsaturated fatty acids (PUFA) play a role in enhancing corpus luteum activity. PUFA also enhance the activity of prostaglandin E 2 (PGE 2) in cattle (Funston, 2004) and PGE 2 acts as a luteotropic hormone, counteracting the luteolytic effects of prostaglandin F 2α (PGF 2α) and thereby supporting the maintenance and function of the corpus luteum. These observations collectively suggest that fatty acids have a significant impact on corpus luteum development and heightened steroidogenic activity in the ovary. Lopes *et al.* (2009) reported improved SPC following the administration of fatty acids. Armstrong *et al.* (1990) also observed better SPC in cattle after supplementation of fatty acids (fishmeal @ 0.8 kg/day) 1.62 vs. 2.31 in the supplemented and non-supplemented group respectively. Ambrose *et al.* (2006) observed an improvement in the conception rate (72.6 vs 47.5%) following supplementation with flaxseed oil compared to sunflower oil (n-3 vs n-6), similar result was also observed by Wathes *et al.* (2007). Sinedino *et al.* (2017) observed higher pregnancy rate (43.6 vs 32.3 %) following supplementation of algae rich in DHA, on day 32. Nazir *et al.* (2013) observed higher pregnancy rate (66.7 vs. 32.1%) in the buffalo following feeding of the flaxseed. Lower embryonic death following feeding of omega-3 fatty acids (Petit and Twagiramungu, 2006).

Blood progesterone level

Progesterone level on day 0 of the experiment was significantly lower in the anoestrus cows *i.e.*, 0.48 ± 0.09 , 0.54 ± 0.12 and 0.69 ± 0.10 ng/ml which increased during the progress of the experiment to 2.42 ± 0.27 , 2.84 ± 0.23 and 3.19 ± 0.32 ng/ml in the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO group cows, respectively (Table 1). Progesterone levels in the anoestrus cows under AN-C group on day 60 (1.41 ± 0.16) and day 90 (2.42 ± 0.27) were significantly ($P < 0.01$) higher than the earlier days level. While in AN-BPF-PO and AN-BPF-PO + BPF-FO group cows progesterone levels increased to >1 ng/ml from day 30 onwards. Significant increases in the progesterone level within the groups were observed after 60 days of the trial, it might be due to the cows become cyclical and pregnant. With positive energy balance, cow shows earlier ovarian

Table 1: Progesterone level (ng/ml) in anoestrus cows supplemented with bypass fat (n=24).

Days of supplementation	Group			Sig. level (P value)
	AN-C	AN-BPF-PO	AN- BPF-PO + BPF-FO	
Day 0	0.48±0.09 ^C	0.54±0.12 ^D	0.69±0.10 ^C	0.371
Day 7	0.63±0.10 ^C	0.60±0.09 ^{CD}	0.65±0.08 ^C	0.932
Day 30	0.81±0.20 ^C	1.13±0.17 ^C	1.01±0.20 ^C	0.489
Day 60	1.41±0.16 ^B	2.33±0.21 ^B	1.99±0.49 ^B	0.165
Day 90	2.42±0.27 ^A	2.84±0.23 ^A	3.19±0.32 ^A	0.177
	P<0.01	P<0.01	P<0.01	

Means with different superscripts in columns (A, B) differed significantly (P<0.01).

activity, ovulation and corpus luteal function (Mattos *et al.*, 2000). Ulfina *et al.* (2015) reported a significant improvement in plasma progesterone concentration (P<0.05) following the feeding of long-chain fatty acids (LCFA) and short-chain fatty acids (SCFA). Gokuldas, (2015) reported that supplementation of PUFA of n-3 class supplemented in the form of fish oil, fish meal, flaxseed improved the progesterone concentration during luteal phase of the estrus cycle in cattle. This increase in progesterone levels might also be associated with a reduced clearance rate from the body, possibly due to the rise in EPA (eicosapentaenoic acid) concentration, as suggested by Mac Laren *et al.* (2006) and Galbreath *et al.* (2008). Plewes *et al.* (2017) reported that EPA and DHA were distributed within the luteal tissue in a dose-dependent manner and increased the lateral mobility of FP receptors and decreased receptors to bind on the plasma membrane and disrupted the luteolytic signalling. These explained the increase in the progesterone level in the supplemented group with the higher conception rate in the current study.

Changes in blood NEFA level

NEFA level ranged from 0.35±0.05 to 0.63±0.04; 0.35±0.01 ±0.06 to 0.94±0.15 and 0.31±0.02 to 0.64±0.05 mmol/l in the AN-C, AN-BPF-PO and AN-BPF-PO + BPF-FO group respectively. Significant reduction plasma NEFA levels were recorded with the progression of the experiment to 90 days in all the three groups of cows. There was a significant reduction observed when NEFA level of day 0 compared with day 14, 21, 56, in the control group; on day 14 to 90 in AN-BPF-PO and on day 14, 21, 63, 77 in AN-BPF-PO + BPF-FO group, respectively. In the present study, it was found that the NEFA level significantly varied between the AN-C, AN-BPF-PO and AN- BPF-PO + BPF-FO groups respectively. NEFA level in the AN-BPF-PO was significantly lower from day 42 to 77 compared to the control group. In the AN-BPF-PO group, NEFA level declined earlier with the progress of the experiment, indicating that bypass fat acted as an energy source (Nirwan *et al.*, 2019). Slow changes in the NEFA level were observed in the AN-BPF-PO + BPF-FO group and this is in accordance with the findings of Juchema *et al.* (2008). Sutaria *et al.* (2022) also recorded that the NEFA level did not differ significantly between the

time points or between the buffaloes suffering from postpartum disorders or not.

CONCLUSION

It was found in the present study that supplementation of palm and fish oil-based bypass fats to the ration of anoestrus cows had resulted in significant reduction in the blood NEFA level, early resumption of cyclicity, improved behavioural estrus, blood progesterone level and conception rate. Supplementation of bypass fats to the ration of dairy cows during early and mid-lactation may be recommended to reduce/manage the incidences of anoestrus in dairy cows.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Institute Research Council of ICAR NDRI, Karnal and supplementation of bypass fat to the ration of dairy cows to enhance productivity is already in practice, does not require any specific approval.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish or preparation of the manuscript.

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