



Strategic supplementation of novel feed supplement improved the lactation and reproductive performance in post-partum dairy cows

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ABSTRACT

The present experiment aimed to establish the efficacy of dietary inclusion of a novel feed supplement on plane of nutrition, milk yield, and its characteristics, reproductive events and circulating concentrations of reproductive hormones in dairy cows during early lactation. Twelve post-partum crossbred lactating cows were equally and randomly assigned to two dietary treatments for 90 days. The cows in CON (control) group were fed on green fodder and wheat straw-based diet with concentrate mixture, whereas, cows in NFS (Novel Feed Supplement) group were fed according to CON group with additional novel feed supplement @0.25% of BW. A comparative study of plane of nutrition revealed that both DCP and TDN were deficient in CON by 12.0 and 18.99%, respectively, however, it was sufficient in NFS group. The daily and total milk yield (kg), 4% FCM, ECM, fat and protein yields (g/d) were improved in NFS than CON group. The average composition of milk showed no significant difference throughout the study. The supplement fed cows had an evident early return of heat (26.20 d in NFS v/s 42.60 d in CON) with enhanced conception rate (66.7% in NFS v/s 50% in CON). The estradiol concentration (pg/ml) was reduced and progesterone concentration (ng/ml) was enhanced in NFS fed cows. In conclusion, incorporation of the novel feed supplement @0.25% of BW significantly improved the plane of nutrition, milk production and reproduction in lactating crossbred cows during the early post-partum period.

Keywords: Cows, Estrogen, Milk yield, Novel feed supplement, Progesterone, Reproduction

Indian livestock production is a predominantly smallholder enterprise that accounts for most of the rural economy. The socio-economic development of the rural population is largely influenced by livestock production. Livestock production is most prevalent among marginal and smallholder farmers in India, and more than 62% of them are directly associated with this sector (Das *et al.* 2020). Therefore, the challenges of maintaining the productivity of dairy farming need to be addressed and resolved with the current perspective.

The complementary action of livestock rearing and green fodder production is creating many challenges for livestock enterprises. These challenges mainly include scarcity of palatable quality fodder, deficiency of specific nutrients along with scarce and unbalanced nutritional practices. According to IGFR Vision 2030, India will face an overall deficit of 65.45% and 24.90% green fodder and dry crop residues, respectively by the year 2030 (IGFR 2011). In the concurrent scenario, the researchers are mainly focusing on fodder-based economical feeding approaches

so as to economize the cost of final livestock products disposal with sustainable production. With an increasing population size and decreasing cultivatable land for fodder production, this enterprise is suffering from tremendous pressure to produce optimally. Consequently, the animals are forced to feed on low-quality crop residue based rations, agro-industrial by products or locally accessible green fodders lacking balanced supply of fermentable energy, degradable protein and minerals to the rumen. Hence, it is reducing the ruminal fermentation and microbial biomass leading to compromised production and reproduction in animals (Uddin *et al.* 2015). Critical supplementation of limiting nutrients to poor quality roughages can efficiently modulate the activity of rumen microbial consortium through a positive associative effect on intake, digestibility and utilization (Yulistiani *et al.* 2015, Wang *et al.* 2019). Significant effect of dietary regime during the transition period on reproductive events in dairy cows through the somatotrophic axis has been noticed (Santos *et al.* 2011).

Keeping in view, a novel feed supplement was formulated which is rich in easily fermentable carbohydrates, degradable protein as well as macro and trace minerals. Hence, the present study investigated the effect of strategic supplementation of a novel feed supplement on the productive and reproductive performance of lactating

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crossbred cows. Further, we hypothesized that novel feed supplement would prevent and alleviate the specific nutrient paucities and production losses in cows, frequently exhibited by lactating animals under common dairy practices.

MATERIALS AND METHODS

Animals, treatments and management: All procedures regarding animal handling and treatments were approved (25/17/2019-CPCSEA-DADF) by the Institutional Animal Ethics Committee and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) in India.

The experiment was undertaken at Farm unit of Livestock Production Management (LPM) Section, ICAR-Indian Veterinary Research Institute, Izatnagar, India. Twelve crossbred (*Bos taurus* × *Bos indicus*) lactating cows (60 days post-partum; 314.29±11.12 kg mean BW; 8.36±0.33 kg milk yield/d) were randomly assigned to CON (control) and NFS (Novel Feed Supplement) groups consisting of six cows in each. The experimental cows in CON group were fed on green oats, berseem and wheat straw-based diet with concentrate mixture (maize: 35; deoiled soybean meal: 15; wheat bran: 50%) as per dairy practices, whereas, cows in NFS group were fed according to CON with additional novel feed supplement (patent application no. 202211032024; dated 03.05.2022) @0.25% of BW. All the cows were offered two equal portions of daily allowance of respective supplements by separate mangers at the time of milking (morning and evening). Green fodder (green oats and berseem) and wheat straw were offered *ab lib*.

The animals were housed in hygienic well-ventilated, roofed and cement-floored sheds with facilities for proper feeding and watering (clean tap water, free choice twice daily). Standard prophylactic measures including deworming for endo and ecto-parasites was followed before the start of experimentation. The ration schedule was adjusted every fortnight after recording the milk yield and body weight of each cow. The feeding trials were continued from 60 day post-partum to 150 day lactation period (total experimental period was 90 days). The feed samples were analysed for proximate principles by the methods of AOAC (2012), and fibre fractions were analysed as per Van Soest

et al. (1991). The chemical composition of feeds offered to the cows is presented in Table 1.

Milk yield and characteristics: Cows were milked twice daily (07:00 and 16:00 hrs) in hygienic manner to arrive milk yield per day. About 100 ml thoroughly mixed milk samples from individual animals were transferred to a screw capped sample bottle with rounded corners (to avoid lodging of the milk solids) at fortnightly intervals. Properly labelled sample bottles were dispatched to laboratory at the earliest and processed immediately. Thoroughly mixed milk samples were analysed for milk fat, SNF, lactose, protein and ash contents using Ultrasonic milk analyzer (Master Classic milk analyser, LM-2). The total solid in the samples were determined by adding SNF and fat content of the milk. 4% FCM and ECM in the milk was calculated as per Gains (1928) and Tyrrell and Reid (1965), respectively.

Reproductive performance: Oestrus detection of all lactating cows was done twice daily during the entire experimental trial period with the help of a trained teaser bull. The post-partum cows in both the groups exhibiting oestrus were inseminated artificially with frozen semen. All the non-return cows were diagnosed for pregnancy by transrectal palpation at 60 d post-insemination. Post-partum reproductive variables such as onset of oestrus, services per conception and conception rate were recorded. The cows were also closely monitored for any post-partum disease/disorders.

Reproductive hormones: The quantitative determination of reproductive hormones, viz. estradiol and progesterone in the serum samples collected at 0, 45 and 90 days of experimental feeding were performed via estradiol (DEH3355) and progesterone (DE1561) ELISA kits (Demeditec Diagnostics GmbH, Germany) as per manufacturer instructions using Microplate spectrophotometer.

Statistical analysis: The data produced in the present experiment was subjected to statistical analysis as per Snedecor and Cochran (2004) using IBM, SPSS (20.0) and means were categorized using Tukey's test. The parameters containing periodic collections were scrutinized by implementing repeated measures protocol via GLM of SPSS; which encompassed between-subjects principal effect of treatments, within-subjects principal effect of period and interaction amid the treatment and

Table 1. Chemical composition of feeds offered (% DM basis)

Attribute	Concentrate mixtures		Wheat straw	Green oats	Berseem
	CON	NFS			
DM	90.19	90.35	92.35	18.85	13.44
OM	94.77	93.06	94.96	89.70	90.40
CP	19.01	25.35	4.33	8.57	17.55
EE	2.05	3.03	1.04	2.89	3.20
TA	5.23	6.94	5.04	10.30	9.60
NDF	40.38	44.54	82.04	65.61	47.29
ADF	11.53	13.07	54.65	42.35	39.62

Abbreviations: CON, Control; NFS, Novel feed supplement; DM, Dry matter; OM, Organic matter; CP, Crude protein; EE, Ether extract; TA, Total ash; NDF, Neutral detergent fibre; ADF, Acid detergent fibre.

period. Independent samples t-test was performed for the parameters not involving any periodic collections. $P < 0.05$ was used to declare the means to be significant unless otherwise stated.

RESULTS AND DISCUSSION

Plane of nutrition: The comparative study of plane of nutrition with Kearn (1982) feeding standard revealed that both DCP (12.0%) and TDN (18.99%) were deficit in CON, however, it was sufficient in NFS group (Fig. 1). The plane of nutrition is largely influenced by the composition of diet. Several researchers have been reported an improved intake of CP and TDN with increasing levels of concentrate mixtures in lactating goats, crossbred heifers and beef cattle (Pereira *et al.* 2007, de Souza Duarte *et al.* 2011, Sultana *et al.* 2012, Mahfuz *et al.* 2018).

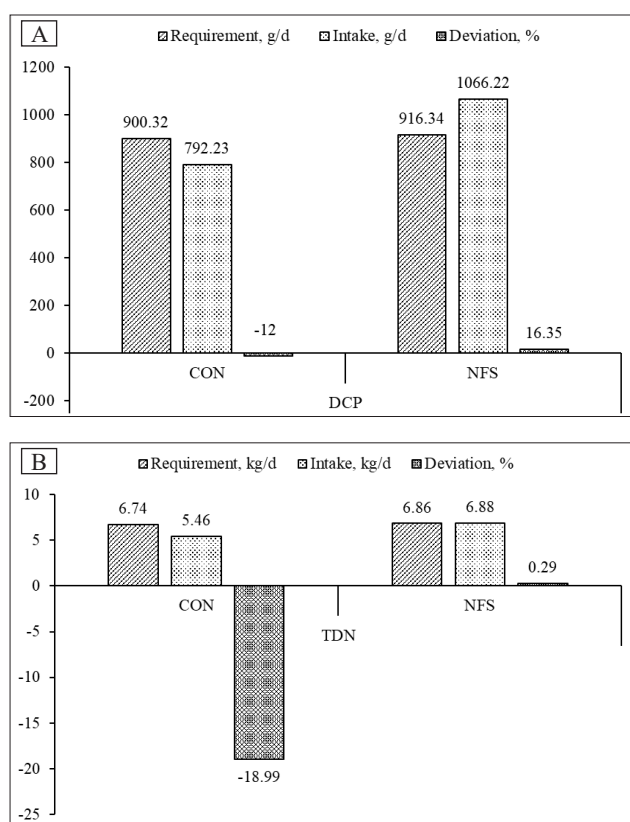


Fig. 1. Comparative plane of nutrition with Kearn (1982) feeding standard of lactating cows supplemented with novel feed supplement (A) DCP, Digestible crude protein and (B) TDN, total digestible nutrient.

Milk yield and characteristics: The daily milk yield (kg) was significantly ($P < 0.001$) higher in NFS than CON group. The total milk production (kg), 4% FCM, ECM, fat and protein yields (g/d) were also followed the similar trend and was higher ($P < 0.001$) in NFS group than CON. The composition of milk such as fat, SNF, total solid, protein, lactose and ash showed no significant difference ($P > 0.05$) between the groups (Table 2). It has been well established that the dietary energy and proteins are the most critical nutrients for optimum lactation performance

Table 2. Effect of novel feed supplement on lactation performance of dairy cows

Attribute	Dietary treatments		P-value
	CON	NFS	
<i>Milk yield</i>			
Daily milk yield, kg/d	8.02±0.20 ^b	9.19±0.16 ^a	0.000
Total milk yield, kg	721.99±28.39 ^b	827.14±24.09 ^a	0.000
4 % FCM, kg/d	9.07±0.23 ^b	11.00±0.19 ^a	0.000
ECM, kg/d	9.38±0.24 ^b	11.31±0.20 ^a	0.000
Fat yield, g/d	390.68±9.90 ^b	488.01±8.57 ^a	0.000
Protein yield, g/d	235.85±5.98 ^b	275.71±4.84 ^a	0.000
<i>Milk composition, %</i>			
Fat	4.87±0.23	5.31±0.24	0.223
SNF	8.13±0.08	8.29±0.06	0.121
Total solid	13.06±0.26	13.54±0.34	0.227
Protein	2.94±0.02	3.00±0.02	0.160
Lactose	4.39±0.13	4.52±0.09	0.115
Ash	0.63±0.01	0.63±0.01	0.810

Abbreviations: CON, basal diet (green fodder and wheat straw-based diet with concentrate mixture); NFS, CON + novel feed supplement @ 0.25% of BW; FCM, Fat Corrected Milk; ECM, Energy Corrected Milk; SNF, Solid Not Fat. ^{a,b}Means in the same row with different superscript differ significantly ($P < 0.001$).

of dairy animals (Wang *et al.* 2014). In agreement with the present results, several researchers have been reported an increased milk yield in dairy cows and goats fed different levels of concentrate mixture (Muñoz *et al.* 2015, Obese *et al.* 2018, Adjorlolo *et al.* 2019). McKay *et al.* (2019) reported significantly higher milk yield, 4% FCM, ECM, fat and protein yields from dairy cows fed barley or maize based concentrate diet as compared to pasture diet alone. Grala *et al.* (2011) also reported an increased milk production, fat, protein, 4% FCM and ECM yields in dairy cows fed concentrate mixture. Zhou *et al.* (2015) reported that daily milk production, 4% FCM, ECM, fat and protein yields were significantly higher for cows fed high energy diet than those fed on low energy diet. Feeding of high energy corn silage increased the ruminal concentration of fermentable carbohydrates and made energy available for milk production (Zhou *et al.* 2015). The improved lactation performance of dairy cows is reported to be closely associated with the continuous supply of rumen fermentable carbohydrates, easily degradable protein and microbial protein to rumen (Wang *et al.* 2014, Zhou *et al.* 2015). Accordingly, the improved lactation performance of crossbred dairy cows fed novel feed supplement may also be due to the increased supply of easily fermentable energy and protein (Chaudhary *et al.* 2021).

Reproductive performance: Data on the reproductive performance of cattle are presented in Table 3. The conception rate in NFS (66.67%) group was considerably improved as compared to CON (50%). The cows fed NFS came into heat (oestrus) following 26.20 d, however, cows in CON group become oestrus after 42.60 d and were inseminated. Following insemination, 33.33 and 16.67% of total animals from CON and NFS groups, respectively were

Table 3. Effect of novel feed supplement on reproductive performance of lactating cows

Attribute	Dietary treatments		P-value
	CON	NFS	
Total no. of animals selected	6	6	-
Total no. of animals confirmed pregnant	3	4	-
No. of animals conceived at first insemination	1 (16.67%)	3 (50%)	-
No. of animals conceived at subsequent insemination	2 (33.33%)	1 (16.67%)	-
No. of animals remained non-responsive	1 (16.67%)	1 (16.67%)	-
First oestrus after start of feeding (days)	42.60±3.83 ^a	26.20±2.29 ^b	0.006
No. of inseminations per conception	1.67±0.33	1.25±0.25	0.352

Abbreviation: CON, basal diet (green fodder and wheat straw-based diet with concentrate mixture); NFS, CON + novel feed supplement @0.25% of BW. ^{a,b}Means in the same row with different superscript differ significantly (P<0.01).

returned to heat again and become pregnant in subsequent inseminations. A total of 16.67% of the animals were non-responsive and did not show any sign of oestrus in both the group. The number of inseminations per conception were 1.67 and 1.25 in CON and NFS, respectively and were comparable (P>0.05) between them. Nutrition also plays an important role in regulating the reproductive performance like postpartum oestrus expression and ovarian functions in animals (Maurya *et al.*, 2010). Good nutrition directly influences the fertility in cattle through supply of essential nutrients needed for gametogenesis and synthesis of blood metabolites related to ovulation and pregnancy (Robinson *et al.* 2006, Königsson *et al.* 2008, Samadi *et al.* 2013). Dairy animals with inadequate nutrition and poor BCS during early post-partum period predisposes to Negative Energy Balance (NEB). This NEB severely affects both productive and reproductive performance by reducing the plasma glucose, insulin and IGF-1 concentrations and increasing the NEFA concentrations (Kawashima *et al.* 2012). NEB delays the involution of uterus, increases the incidences of anoestrus, days open, delays or inhibit the ovulation rate by inhibiting LH surge, reduces ovulatory ovarian follicular functions, increases calving interval as well as the number of services per conception (Robinson *et al.* 2006, Peter *et al.* 2009, Kafi and Mirzaei 2010, Drackley and Cardoso 2014). The inhibition of LH pulse and decreased blood concentrations of glucose, insulin and IGF-1 results in low oestradiol concentration which is essential for initiation of gonadotrophin surge and successive ovulation (Wathes *et al.* 2007, Walsh *et al.* 2011, Soca *et al.* 2014). In accordance with the present results, Adjorlolo *et al.* (2019) reported that cows supplemented with 2.5 kg of concentrate per day had significantly less proportion of non-cyclic animals (20%) than non-supplemented group (55%). They proposed that higher

plasma protein concentrations in supplemented group indicated improved nutritional status, therefore, increased conception rate in cattle (Robinson *et al.* 2006, Almeida 2017). Tewari *et al.* (2014) also reported that onset of estrus and conception rate were significantly higher following strategic nutrient supplementation to heifers and lactating cows under field condition. The role of minerals to maximise the reproductive efficiency of dairy animals has already been established by several workers (Chester-Jones *et al.* 2013, Ghosh *et al.* 2016). In agreement with our results, several workers have reported a significant reduction in first oestrus post-feeding (days) and number of services per conception following supplementation of mineral mixture in problematic dairy animals (Mohapatra *et al.* 2012, Agrawalla *et al.* 2017).

Reproductive hormones: The estradiol concentration (pg/ml) of cattle was significantly (P<0.05) lower and progesterone concentration (ng/ml) was significantly (P<0.05) higher in NFS relative to CON group (Table 4). Progesterone plays an important role in the reproductive events related to establishment and maintenance of pregnancy (Lonergan *et al.* 2016, Lonergan and Sánchez 2020). A reduced circulating progesterone concentration during growth of the ovulatory follicle decreases fertility, however, reduced circulating progesterone concentration after ovulation have been implicated as a causative factor for decreased conceptus growth, decreased production of interferon- τ and lower pregnancy rates in high-yielding dairy cows (Lonergan and Sánchez 2020). It has been reported that NEB reduces serum progesterone concentration and hence, fertility in dairy animals (Butler 2005). The concentration of serum progesterone and estrogen can be altered due to deficiency of micro-nutrients. Trace minerals such as copper and zinc are linked to reproductive hormones, viz. progesterone and estradiol (Prasad *et al.* 1989) and are

Table 4. Effect of novel feed supplement on reproductive hormones in lactating cows

Hormone	Dietary treatments		SEM	P-value [†]		
	CON	NFS		T	P	TxP
Estradiol, pg/ml	951.10 ^a	616.31 ^b	59.03	0.001	0.023	0.159
Progesterone, ng/ml	2.93 ^b	7.14 ^a	1.00	0.008	0.001	0.128

Abbreviation: CON, basal diet (green fodder and wheat straw-based diet with concentrate mixture); NFS, CON + novel feed supplement @0.25% of BW; SEM, Standard error of means. [†]Significant effects of the dietary treatments (T), period (P) and their interactions (T×P). ^{a,b}Means in the same row with different superscript differ significantly (P<0.05).

the activators of specific enzyme systems that support in maintaining the activity of these hormones in the blood (Georgievskii 1982). Hence, deficiency of trace minerals adversely affects the reproductive performance of the farm animals (Bearden *et al.* 2004). Godara *et al.* (2016) and Joshi *et al.* (2019) observed significantly higher circulating concentration of progesterone in mineral supplemented problematic crossbred cattle and buffaloes than their non-supplemented counterparts, however, no significant change was observed in plasma estrogen level. Rajala-Schultz and Saville (2003) reported improved serum concentration of progesterone in cows following supplementation of high energy diet as compared to cows fed low energy diet. Khan *et al.* (2018) reported significantly higher progesterone concentration in early lactating cows fed high energy diet. The reason behind the lower plasma progesterone concentration in non-supplemented cattle may be due to the lower steroidogenesis associated with altered enzymatic activity because of lower plasma trace mineral concentrations (Godara *et al.* 2016). The higher concentration of serum progesterone hormone in novel feed supplement fed cows is attributed to the higher conception rate in these groups as evident from the study. This also reflects the re-establishment of normal cyclicity and luteal phase in animals following supplementation (Joshi *et al.* 2019).

Based on the results, it can be concluded that dietary supplementation of novel feed supplement @0.25% of body weight significantly improved the plane of nutrition, daily and total milk production, 4% FCM, ECM, fat, and protein yields as well as overall reproductive performance of lactating crossbred cattle during early lactation.

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