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RESEARCH ARTICLE

Assessing the revitalization of postpartum performance and health in dairy cows with the novel feed supplement

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Abstract: The current study investigated the effects of a novel feed supplement (NFS) on nutrient intake, body weight, body condition score, energy balance, metabolic profile, and mineral status in dairy cows during early lactation. A total of 12 post-partum crossbred lactating cows were randomly divided into two groups (n=6): a control group (CON) and a novel feed supplement group (NFS), with experimental feeding conducted over a 90-day period. The CON group was fed a diet consisting of green fodder, wheat straw, and a concentrate mixture, whereas the NFS group received the same diet supplemented with a novel feed supplement @ 0.25% of body weight. Results showed that cows in the NFS group had a higher voluntary feed intake compared to the CON group. Body weights and body condition scores remained comparable between both groups. Cows fed with NFS had significantly higher serum glucose and lower serum non-esterified fatty acids (NEFA) levels than those in the CON group. Additionally, haemoglobin, hematocrit, serum total protein, albumin, and urea levels were significantly elevated in the NFS group, while serum ALT levels were reduced compared to the CON group. Serum levels of key minerals (Ca, iP, Zn, Cu, Fe, and Mn) were also enhanced in the NFS group. In conclusion, dietary inclusion of the novel feed supplement at 0.25% of body weight significantly improved nutrient intake, energy balance, metabolic profile, and mineral status in lactating crossbred cows during the early postpartum period.

Keywords: Dairy cows, Health, Negative energy balance, NEFA, Novel feed supplement

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Introduction

India's rural lifestyle is predominantly agriculture-based, with livestock playing a vital role in its economy. The country possesses vast genetic resources of livestock, largely held by landless or marginal farmers, with a relatively small share owned by organized sectors. A major challenge for India's livestock sector is to sustain its large livestock population alongside meeting the food demands of its human population. This challenge is exacerbated by declining green fodder availability, critical nutrient deficiencies, and unbalanced feeding practices. With diminishing green forage production, animals are increasingly fed on poor-quality roughages, locally available feed resources, and tree leaves. These low-quality roughages and unbalanced feeding practices deprive high-producing dairy animals of essential fermentable energy, degradable protein, and minerals, leading to reduced ruminal fermentation, lower microbial biomass production, and consequently, decreased productivity, reproduction, and health (Uddin et al. 2015).

During early lactation, dairy animals experience a negative energy balance (NEB), as energy demands for lactation surpass dietary energy intake. Postpartum NEB is characterized by low levels of blood glucose, insulin, and IGF-1, alongside high concentrations of ketone bodies and non-esterified fatty acids (NEFA) (Sammad et al. 2022). Thus, ensuring adequate feed intake and energy balance during the peri-parturient period is essential for maintaining cow health, milk production, and timely resumption of post-calving reproductive functions. Addressing the early postpartum period, the effects of NEB can be mitigated by strategic supplementation of limiting nutrients to support poor-quality roughages and correct unbalanced diets. This approach can effectively enhance rumen microbial activity, promoting intake, digestibility, and nutrient utilization (Yulistiani et al. 2015; Wang et al. 2019).

In line with this objective, a customized novel feed supplement was developed, incorporating fermentable carbohydrates, degradable protein, and essential macro and trace minerals to correct nutritional imbalances inherent in roughage-based diets. This formulation aims to enhance rumen microbial efficiency, improve nutrient utilization, and support metabolic resilience during early lactation. The present study explores the effects of dietary supplementation with this novel feed supplement on voluntary feed intake, body weight, body condition score, energy balance, hematological and biochemical profiles, and serum mineral status in lactating cows during the early postpartum period.

Materials and Methods

All the experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India (No. F. 26-1/2018-19/J.D.(R) dated 07/10/2019).

Animals, treatments and management

The experiment was conducted at the Farm Unit of the Livestock Production Management Section, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. Twelve crossbred lactating cows (Bos taurus x Bos indicus) 60 days postpartum, with an average body weight of 314.29 ± 11.12 kg and an average daily milk yield of 8.36 ± 0.33 kg, were randomly divided into two groups: the control group (CON) and the Novel Feed Supplement group (NFS), with six cows in each. The CON group was fed a green oat, berseem, and wheat straw-based diet supplemented with a concentrate mixture (maize: 35%, deoiled soybean meal: 15%, wheat bran: 50%) as per the dairy practices. The NFS group received the same diet as the CON group with an additional novel feed supplement (patent application number 202211032024, dated 03.06.2022) at 0.25% of body weight. Each cow's daily supplement allowance was divided into two portions and provided in separate mangers at morning and evening milking times. Green fodder (oats and berseem) and wheat straw were offered *ad libitum*.

The cows were housed in a well-ventilated, roofed, and cement-floored shed with clean feeding and watering facilities, with free access to clean tap water twice daily. Standard prophylactic measures, including deworming for endo and ectoparasites, were applied before starting the experiment. The ration schedule was adjusted biweekly based on each cow's milk yield and body weight. The feeding trial ran from 60 to 150 days postpartum, covering a total experimental period of 90 days. Feed samples were analyzed for proximate principles according to AOAC (2012) methods, and fiber fractions were determined as per Van Soest et al. (1991). The chemical composition of feeds offered to the cows is presented in Table 1.

Body weight and body condition score: Body weight (BW) and body condition score (BCS) of each animal were monitored biweekly. BCS was assessed using a visual method developed by Edmonson et al. (1989), examining key body areas for changes. The scoring scale ranged from 1 (emaciated) to 5 (obese), with 0.5-unit increments.

Clinical chemistry parameters

Blood samples were collected from each cow at 0, 45, and 90 days of feeding via jugular venipuncture, using sterilized needles early in the morning before feeding. Serum samples were extracted and stored at -20°C for later analysis. An additional 2 mL of blood was treated with EDTA (1 mg/mL) for hematological analysis.

Hemoglobin and hematocrit concentrations were measured using the cyanmethemoglobin method (Dacie and Lewis, 1969) and the microhematocrit method, respectively. Serum biochemical parameters were analyzed spectrophotometrically (Multiskan™ FC Microplate Photometer, Thermo Scientific Ltd.) using diagnostic kits from Coral Clinical Systems, Tulip Diagnostics (P) Ltd., India, as per the manufacturer's instructions. Serum NEFA levels were determined using a competitive ELISA-based Human NEFA (Nesfatin-1) ELISA Kit (ImmunoTag™).

Serum minerals

Major minerals (Ca and iP) were analyzed spectrophotometrically with diagnostic kits from Coral Clinical Systems.

Trace minerals (Cu, Zn, Fe, and Mn) were quantified using an Atomic Absorption Spectrophotometer (Electronics Corporation of India Ltd., Model No. 4141). For this, 0.5ml of serum samples were digested in 10 ml of triple acid consisting of nitric acid, sulphuric acid and perchloric acid in 4:2:1 ratio. Suitable working standards from standard stock solution (1000 µg/ml) was prepared with the help of triple glass distilled water. After that, a series of standard solutions were prepared from the working standard to plot a calibration curve between the absorbance and their concentration. A blank was also used to minimise the errors while preparing the mineral extracts. The operating protocol for estimation of the trace minerals is presented below:

SN	Particulars	Zn	Cu	Fe	Mn
1	Wavelength (nm)	213.9	324.7	248.3	279.5
2	Slit setting (nm)	1.5	0.5	0.2	0.2
3	Light source	Hollow cathode lamp			
4	Flame type	Air–Acetylene flame			

Statistical analysis

Data collected in this study were analyzed using the methods described by Snedecor and Cochran (2004) with IBM SPSS 20.0. Independent samples t-tests assessed nutrient intake, body weight, and BCS, while parameters involving repeated measurements were analyzed using a repeated measures protocol within the GLM of SPSS (IBM SPSS Statistics Version 20). This included the between-subjects effect of treatments, the within-subjects effect of time period, and treatment-by-period interactions. Means were categorized using Tukey's test, with statistical significance set at $P<0.05$ unless otherwise noted.

Results and Discussion

Feed intake and body weight change

Voluntary feed intake (kg/d or $gkg^{-1}W^{0.75}$) over the 90-day experimental period was significantly higher (P<0.05) in the NFS group compared to the CON group (Table 2). Body weight (BW, kg), average daily gain (ADG, g), total body weight gain (kg), and body condition score (BCS) remained comparable (P>0.05) between the CON and NFS groups throughout the feeding period. Nutrient intake is primarily influenced by diet composition, palatability, fiber degradation, and digesta flow rate.

These findings align with Zhou et al. (2015), who observed significantly higher DMI and organic matter (OM) intake in cows fed a high-energy diet compared to those on a low-energy diet. Wang et al. (2023) demonstrated that high-concentrate diets significantly improved nutrient digestion, with the liver adapting by regulating genes involved in nutrient metabolism. Similarly, in buffaloes and sheep, feeding high-CP concentrates supplemented with urea significantly improved DM intake, with the inclusion of non-protein nitrogen (NPN) compounds shown to enhance rumen fermentation and digestibility (Sweeny et al. 2014; Kang et al. 2015). Further supporting this, Uddin et al. (2015) identified a strong positive correlation between DM intake and microbial growth in the rumen.

An associative effect among forages has been observed, where adding high-nitrogen supplements to low-quality roughages enhances microbial activity in the rumen, promoting increased intake and digestion (Wahyono et al. 2022). This associative effect may have created an optimal environment for improved rumen fermentation, leading to the observed increase in nutrient intake with novel feed supplementation in this study. Consistent with these findings, Adjorlolo et al. (2019) reported no significant differences in body weight between cows fed 2.5 kg of concentrate daily and non-supplemented cows. However, contrary to our findings, Xia et al. (2018) reported that dietary CP level exerted a significant positive effect on the final body weight, average daily gain and gain-to-feed ratio of the bulls.

Clinical chemistry parameters

The haematological indices *viz.,* Hb (gd/L) and haematocrit (%) were significantly higher (P<0.01) in cows receiving the NFS compared to the CON group (Table 3). Cows fed NFS also showed

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

Attributes	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

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

Table: 2 Effect of novel feed supplement on nutrient intake in lactating cows

Variables	Dietary treatments		P value
	CON	NFS	
Nutrient intake ($gkg^{-1}W^{0.75}$)			
DM (kg/d)	9.77±0.47[b]	11.40±0.31[a]	0.015
DM	127.95±1.82[b]	147.56±3.56[a]	0.001
OM	119.21±2.68[b]	136.75±5.35[a]	0.016
CP	17.41±1.03[b]	20.86±1.00[a]	0.037
DCP	10.50±0.62[b]	13.86±0.67[a]	0.004
TDN	72.25±3.67[b]	89.30±3.57[a]	0.008
Nutrient density (%)			
DCP	8.19±0.44[b]	9.37±0.28[a]	0.047
TDN	54.40±1.03[b]	60.43±1.36[a]	0.005

[ab]Mean±SE with different superscript in a row differ significantly. ADG, Average daily gain; DCP, Digestible crude protein; TDN, Total digestible nutrient.

Table: 3 Effect of novel feed supplement on blood-biochemical parameters in lactating cows

Treatments	Periods (d)			Treatment mean	SEM	P value*		
	0	45	90			T	P	TxP
Haemoglobin, g/dL								
CON	11.35	12.24	12.79	12.13^{B}				
NFS	11.56	13.46	13.45	12.82^{A}	0.18	0.009	0.000	0.264
Period mean	11.45^{Y}	12.85^{X}	13.12^{X}					
Haematocrit, %								
CON	34.18	36.48	38.49	36.38^{B}				
NFS	35.02	41.52	41.43	39.29^{A}	0.62	0.001	0.000	0.131
Period mean	34.60^{Y}	39.00^{X}	39.91^{X}					
Serum glucose, mg/dL								
CON	50.93^{c}	53.97^{c}	57.48^{bc}	54.13^{B}				
NFS	50.69^{c}	62.62^{ab}	71.20^{a}	61.50^{A}	1.44	0.001	0.000	0.005
Period mean	50.81^{Z}	58.30^{Y}	64.34^{X}					
NEFA, ng/mL								
CON	11.18^{a}	10.60^{a}	9.92^{a}	10.57^{A}				
NFS	11.39^{a}	6.06^{b}	5.18^{b}	7.54^{B}	0.50	0.000	0.000	0.000
Period mean	11.29^{X}	8.33^{X}	7.55^{Y}					
Total protein, g/dL								
CON	6.35	6.93	6.91	6.73^{B}				
NFS	6.85	7.57	7.92	7.45^{A}	0.15	0.008	0.031	0.707
Period mean	6.60^{Y}	7.25^{XY}	7.41^{X}					
Albumin, g/dL								
CON	2.95	2.90	3.09	2.98^{B}				
NFS	2.93	3.27	3.55	3.25^{A}	0.07	0.036	0.042	0.253
Period mean	2.94^{Y}	3.08^{XY}	3.32^{X}					
Globulin, g/dL								
CON	3.39	4.03	3.83	3.75				
NFS	3.91	4.30	4.37	4.20	0.14	0.118	0.270	0.903
Period mean	3.65	4.17	4.10					
A:G ratio								
CON	0.91	0.74	0.83	0.83				
NFS	0.89	0.77	0.84	0.84	0.04	0.930	0.456	0.976
Period mean	0.90	0.76	0.84					
Urea, mg/dL								
CON	29.34^{cd}	29.37^{cd}	30.91^{bc}	29.87^{B}				
NFS	28.76^{d}	32.79^{ab}	34.80^{a}	32.12^{A}	0.41	0.000	0.000	0.000
Period mean	29.05^{Z}	31.08^{Y}	32.85^{X}					
Total-cholesterol, mg/dL								
CON	128.46	126.60	127.31	127.46				
NFS	133.21	134.17	129.36	132.24	3.59	0.546	0.962	0.959
Period mean	130.83	130.39	128.33					
AST, U/L								
CON	83.50	84.42	83.79	83.91				
NFS	83.43	83.70	82.51	83.22	0.51	0.531	0.787	0.904
Period mean	83.47	84.06	83.15					
ALT, U/L								
CON	21.84^{ab}	22.55^{a}	23.35^{a}	22.58^{A}				
NFS	21.74^{ab}	19.87^{bc}	17.91^{c}	19.84^{B}	0.40	0.000	0.146	0.000
Period mean	21.79	21.21	20.63					

$^{AB, abcd, XYZ}$Mean values with different superscript differ significantly. *Significant effects of the dietary treatments (T), period (P) and their interactions (T×P). CON, Control; NFS, Novel feed supplement; SEM, Standard error of mean; NEFA, Nonesterified fatty acids, AST, Aspartate transaminase; ALT, Alanine transaminase.

elevated serum glucose levels (mg/dL; P<0.01) and reduced serum NEFA concentrations (ng/mL; P<0.001) relative to cows on the CON diet (Table 3). Additionally, serum total protein, albumin, and urea concentrations were significantly higher (P<0.05) in the NFS group than in the CON group, while serum ALT (U/L) was notably decreased (P<0.001) in cows fed NFS. Levels of serum globulin, A:G ratio, total cholesterol and AST were comparable (P>0.05) between the treatment groups (Table 3).

The observed Hb and haematocrit values align with the findings of Singh et al. (2013), who reported significantly increased Hb and haematocrit concentrations with higher dietary energy levels in lambs. Plasma glucose and NEFA are primary circulating metabolites for assessing animals' energy status. NEB initiates fat mobilization from adipose tissue, leading to increased circulating NEFA in dairy animals. Consequently, NEFA serves as a marker of under-nutrition severity and NEB. NEB is often characterized by reduced blood glucose, insulin, IGF-1, and elevated ketone bodies and NEFA concentrations (Sammad et al. 2022). Consistent with our findings, Yang et al. (2022) reported that cows on higher concentrate diets exhibited lower plasma NEFA and BHBA concentrations, thereby improving energy balance. Cows receiving higher grain supplementation exhibited increased basal plasma glucose levels (Marett et al. 2015). Schöbitz et al. (2013) also observed reduced plasma NEFA in dairy cows receiving 3–6 kg/day of concentrate compared to non-supplemented cows. The increased serum glucose concentration in the NFS group likely indicates an improved energy status, possibly due to higher feed intake and the readily fermentable energy provided by the supplement. The lower serum NEFA levels in NFS-fed cows further suggest an enhanced energy balance in lactating crossbred cattle.

Protein-rich concentrates and urea molasses mineral blocks (UMMB) have been reported to improve total protein and albumin levels in lambs (Muralidharan et al. 2015). Adjorlolo et al. (2019) found that cows supplemented with 2.5 kg of concentrate daily had significantly higher total protein than non-supplemented cows. Amanlou et al. (2017) similarly reported higher serum total protein and albumin in Holstein cows on diets with increased crude protein (CP) levels. The elevated total protein in the NFS-supplemented group could be due to increased amino acid absorption stemming from enhanced microbial protein synthesis, facilitated by the additional nitrogen from the supplement (Adjorlolo et al. 2019).

Table: 4 Effect of novel feed supplement on mineral status in lactating cows

Treatments	Periods (d)			Treatment mean	SEM	P-value		
	0	45	90			T	P	TxP
Calcium, mg/dL								
CON	9.13^{b}	9.40^{b}	9.85^{b}	9.46^{B}				
NFS	9.10^{b}	10.94^{a}	11.65^{a}	10.56^{A}	0.18	0.000	0.000	0.000
Period mean	9.12^{Z}	10.17^{Y}	10.75^{X}					
i-Phosphorus, mg/dL								
CON	5.52^{d}	5.99^{b}	5.93^{bc}	5.81^{B}				
NFS	5.55^{cd}	6.43^{a}	6.49^{a}	6.16^{A}	0.07	0.000	0.000	0.014
Period mean	5.54^{Y}	6.21^{X}	6.21^{X}					
Zinc, µg/mL								
CON	0.92^{b}	0.91^{b}	0.90^{b}	0.91^{B}				
NFS	0.91^{b}	1.15^{ab}	1.39^{a}	1.15^{A}	0.04	0.001	0.032	0.018
Period mean	0.92^{Y}	1.03^{XY}	1.14^{X}					
Copper, µg/mL								
CON	0.95^{b}	0.94^{b}	0.95^{b}	0.95^{B}				
NFS	0.94^{b}	1.10^{ab}	1.24^{a}	1.09^{A}	0.04	0.007	0.001	0.007
Period mean	0.94^{Y}	1.02^{XY}	1.09^{X}					
Iron, µg/mL								
CON	3.71^{bc}	3.63^{c}	3.61^{c}	3.65^{B}				
NFS	3.70^{bc}	4.54^{b}	5.35^{a}	4.53^{A}	0.13	0.000	0.002	0.001
Period mean	3.70^{Y}	4.09^{XY}	4.48^{X}					
Manganese, µg/mL								
CON	0.30^{b}	0.29^{b}	0.29^{b}	0.29^{B}				
NFS	0.30^{b}	0.38^{ab}	0.44^{a}	0.38^{A}	0.01	0.000	0.044	0.011
Period mean	0.30^{Y}	0.34^{XY}	0.36^{X}					

$^{AB,\ abcd,\ XYZ}$Mean values with different superscript differ significantly. *Significant effects of the dietary treatments (T), period (P) and their interactions (T×P). CON, Control; NFS, Novel feed supplement; SEM, Standard error of mean

Blood urea nitrogen (BUN) serves as a useful metabolic indicator for evaluating short-term nitrogen utilization and nutritional status in dairy animals (Islam et al. 2019). Studies have shown higher blood urea concentrations in animals fed higher dietary CP, correlating with dietary protein intake (Amanlou et al. 2017). Muralidharan et al. (2015) also reported significantly elevated blood urea-N in lambs supplemented with protein-rich concentrate and UMMB. In dairy heifers, those fed protein-rich diets displayed higher serum urea and blood urea-N than non-supplemented heifers (Islam et al. 2019). Xia et al (2018) showed that blood urea nitrogen increased linearly with an increase in dietary CP level in Holstein bulls. Spek et al. (2013) observed approximately 68% higher plasma urea-N in cows on high-protein diets, noting a positive correlation between dietary protein levels and plasma urea-N. They suggested that increased dietary protein promotes ruminal NH_3 absorption into the blood, enhancing urea synthesis in the liver and expanding the plasma urea pool, which may explain the elevated plasma urea in NFS-fed cattle due to the supplement's high rumen-degradable protein content.

Serum minerals

Serum levels of major minerals, *viz.,* Ca and iP (mg/dL), were significantly higher (P<0.001) in cows fed the NFS compared to the CON group. Similarly, serum concentrations of trace minerals, including Zn, Cu, Fe, and Mn (µg/mL), were also significantly elevated (P<0.01) in the NFS group (Table 4). These results align with previous studies showing that dietary inclusion of area-specific mineral mixtures significantly improves serum macro and trace mineral levels in both healthy and mineral-deficient dairy animals (Satapathy et al. 2016; Sahoo et al. 2017). Muralidharan et al. (2015) similarly observed significantly higher serum Ca and iP levels in lambs supplemented with a concentrate mixture. The increase in serum mineral levels in the present study may be attributed to the mineral-rich composition of the novel feed supplement, which likely contributed to the improved mineral status of the cows (Chaudhary et al. 2021).

Conclusion

Based on these findings, it can be concluded that dietary inclusion of the novel feed supplement at 0.25% of body weight significantly enhances nutrient intake, energy balance, metabolic profile, and mineral status in lactating crossbred cattle during the early lactation period.

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