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EFFECT OF STRATEGIC SUPPLEMENTATION OF A NOVEL FEED SUPPLEMENT ON ZOO-TECHNICAL ATTRIBUTES, METABOLIC PROFILE, PRODUCTION AND REPRODUCTION PERFORMANCE OF BUFFALOES

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ABSTRACT

The present experiment aimed to establish the efficacy of a novel feed supplement (NFS) on nutrient intake, energy status, metabolic profile, milk yield, reproductive performance and its socio-economic impact on problematic post-partum buffaloes under field condition. Twenty-four post-partum buffaloes were randomly and equally assigned to CON and NFS groups for 120 days of experimental feeding period. The animals in CON group were fed on basal roughages and concentrate mixture 20% of DMI as per the farmers' practices, whereas buffaloes in NFS group were fed according to CON with additional novel feed supplement 0.25% of BW. The voluntary feed intake and average daily gain in body weight were increased in NFS than CON group. The buffaloes fed NFS diet exhibited higher Hb, haematocrit, serum glucose and lower NEFA. Serum minerals (Ca, iP, Zn, Cu, Fe, Mn), metabolic hormones (T_3, T_4, GH, IGF-1) and total antioxidant capacity were enhanced in NFS group. The milk yield and conception rate were also improved significantly following strategic supplementation of NFS. Analyses of cost benefit of milk yield revelled that the benefit-cost ratio was improved in NFS fed buffaloes. Thus, it can be concluded that strategic supplementation the novel feed supplement 0.25% of BW significantly improved the nutrient intake, energy balance, metabolic profile, milk yield and reproduction performance of problematic buffaloes under field condition during early post-partum period.

Keywords: *Bubalus bubalis*, buffaloes, conception rate, milk yield, negative energy balance, novel feed supplement

INTRODUCTION

Buffalo is an important livestock resource in many countries including India. They also contribute a significant role in the total milk production of the country. However, the productive

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and reproductive efficiency of buffaloes get compromised majorly because of poor genotype and nutrition. Poor nutrition has been identified as a major constraint in sustaining the animal's performance under field condition and signifies a great loss in both productive and reproductive efficiency. This also contributes to different unwanted ailments and disease conditions amongst which negative energy balance (NEB) is most common during transition and initial lactation period where energy expenditure in lactation exceeds energy intake from the diet (Singh *et al*., 2020). This condition remarkably enhances the release of certain metabolic stressors like NEFA, BHBA etc. Post-partum NEB is characterized by low blood glucose, insulin and IGF-1 along with high ketone bodies and NEFA concentrations (Sammad *et al*., 2022). To avoid the condition of NEB, timely corrective management strategies must be implicated before the onset of the production stage of dairy animals (Caja *et al*., 2016). It is advisable to adopt a management strategy that encourages a reduction in elevated concentration of serum ketone bodies and NEFA by improved DMI (Chapinal *et al*., 2011; McArt *et al*., 2013).

India is experiencing a high gap between the availability and demand of certain feedstuffs. Singh *et al.* (2021) reviewed that India is presently facing a deficit of 35% green forages, 26% dry forages and 41% deficit concentrate feed ingredients. This deficiency is mainly because of continuously increasing livestock and human population, reduced forage cultivatable land and increased demand and competition for food resources. To sustain the livestock production, nutrients available for animals should be assimilated into their body to meet the different production requirements and other physiological activities (Singh *et al*., 2021). Therefore, adequate feed intake and energy balance during the peri-parturient period is of paramount importance for health, milk production and early resumption of ovarian cyclicity in diary animals. An energy deficit diet will promote deamination of amino acids leading to fermentation of its carbon skeleton, however, energy adequate diet will promote transamination of amino acids and microbial protein synthesis (Bach *et al*., 2005). Diet containing optimum rumen degradable protein promotes rumen digestion, microbial growth and the production of microbial protein. Therefore, there is a need to prepare a diet by selecting ingredients that meet the sustainability criteria and positively enhance the livestock production (Makkar and Ankers, 2014).

Therefore, targeting to the early post-partum period, the effect of NEB can be reversed by critical supplementation of limiting nutrients to poor quality roughages and unbalanced feeding regime. This may efficiently modulate the activity of rumen microbial consortium through a positive associative effect on intake, digestibility and utilization of nutrients (Wang *et al*., 2019; Yulistiani *et al*., 2015). Keeping in view, novel feed supplement rich in easily fermentable carbohydrates, degradable protein as well as macro and trace minerals has been formulated. Furthermore, the present study investigated the effect of strategic supplementation of novel feed supplement on zoo-technical attributes, energy status, metabolic profile, milk yield, reproductive performance and its socio-economic impact on problematic post-partum buffaloes under field condition.

MATERIALS AND METHODS

Animals, treatments and management

An *on-farm* trial was carried out to explore the efficacy of NFS on the performance of buffaloes. Problematic buffaloes (N=24, 60 to 70 days post-partum) from Bhojipura and Dabhora villages of Bareilly district of Uttar Pradesh Province, India were selected with the help of participating farmers and were randomly assigned to CON (control) and NFS (Novel Feed Supplement) groups consisting of 12 buffaloes in each group. The animals in CON group were fed on basal roughages (wheat/paddy straw and sugarcane top) and concentrate mixture (wheat bran: 85 and mustard oil cake: 15 kg) 20% of DMI as per the farmers' practices, whereas buffaloes 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. The duration of the study was of 120-day lactation period during which the response to the dietary intervention was ascertained. The buffaloes were individually offered daily weighed quantities of their respective concentrate mixtures and cereal straw with sugarcane top *ad libitum* as per schedule by participating farmers. 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.

Body weight and body condition score

Body weight (BW) and body condition score (BCS) of individual animal was monitored at start, mid and end of experimental period. Body weight of the buffaloes was calculated from their heart girth and body length measurements by Shaffer's formula. The BCS of the buffaloes were performed using a procedure 5 points' scale (Edmonson *et al*., 1989).

Clinical chemistry

About 6 ml blood from the buffaloes (6 buffaloes per treatment) were collected at the end of experimental feeding by jugular venepuncture with the help of clean sterilized needle early in the morning before feeding. Serum samples were harvested and stored at -20°C for further analysis. Additional 2.0 ml of the sample was added with EDTA (1 $mgml^{-1}$) in vials for the haematological examination. The Hb and haematocrit concentrations were assessed by cyanomethaemoglobin and microhematocrit methods, respectively. All the serum biochemical parameters were assessed spectrophotometrically (Multiskan™ FC Microplate Photometer, Thermo Scientific Ltd.) with the help of diagnostic kits manufactured by Coral Clinical systems, a division of Tulip Diagnostics (P) Ltd, India following manufacturer's instructions. The serum NEFA was estimated using competitive-ELISA based Human NEFA (Nesfatin-1) ELISA Kit (ImmunoTag™).

Serum minerals

The major minerals (Ca and iP) were assessed spectrophotometrically (Multiskan™ FC Microplate Photometer, Thermo Scientific Ltd.) with the help of diagnostic kits manufactured by Coral Clinical systems, a division of Tulip Diagnostics (P) Ltd, India following manufacturer's instructions. The serum trace minerals (Cu, Zn, Fe and Mn) were assessed using Atomic Absorbance Spectrophotometer (Electronics Corporation of India Ltd., Hyderabad, India, Model No. 4141).

Milk yield and composition

Daily milk yield was recorded until 120

days of lactation period. About 100 ml thoroughly mixed milk samples from individual buffaloes was 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 Gaines (1928); Tyrrell and Reid (1965), respectively.

Reproductive performance

The buffaloes were observed regularly for symptoms of oestrus. The buffaloes exhibiting oestrus were inseminated with frozen semen. All the non-return buffaloes were diagnosed for pregnancy by transrectal palpation at 60 day of insemination. Post-partum reproductive variables like onset of oestrus, services per conception and conception rate were recorded. The buffaloes were also closely monitored for any post-partum disease/disorders.

Serum hormones and total antioxidant capacity assay

The serum thyroid hormones (T_3 and T_4) were estimated using UBI MAGIWEL™ (United Biotech Inc., USA) ELISA kits. The GH and IGF-1 assay in the serum samples were performed using Bovine GH and Bovine IGF-1 ELISA kits (GenAsia, China and RayBiotech, Georgia), respectively. The estradiol and progesterone assay in the serum samples were done using estradiol and progesterone ELISA Kit (Demeditec Diagnostics GmbH, Germany), respectively. The total antioxidant capacity (TAC) assay was done using Total Antioxidant Capacity Assay Kit (Elabscience Biotechnology Inc., USA).

Statistical analysis

The data produced in the present experiment was subjected to statistical analysis using IBM SPSS statistics software (Chicago, USA). Independent samples t-test was used to assess different parameters related to production and reproduction. The mean ± SE were categorized using Tukey's test and $P<0.05$ was used to declare the significance.

RESULTS AND DISCUSSIONS

Feed intake and body weight change

The voluntary feed intake (kgd^{-1} or $gkg^{-1}W^{0.75}$) and ADG (gd^{-1}) were significantly ($P<0.01$) higher in NFS than CON group, however, the BW and BCS remained comparable ($P>0.05$) in both CON and NFS groups (Table 2 and Figure 1). In accordance with the present results, a significantly higher intake of DM, OM, TDN and CP in cows, lactating goats and beef cattle fed higher levels of concentrate mixtures or high energy diet have been reported (Duarte *et al*., 2011; Sultana *et al*., 2012; Zhou *et al*., 2015). Furthermore, DM intake has been reported to significantly improve in buffaloes and sheep fed concentrate containing higher level of CP with additional urea and hypothesized that a proportionate inclusion of NPN compound contributed to improved rumen fermentation and digestibility (Kang *et al*., 2015; Sweeny *et al*., 2014). A strong positive correlation between DM intake and microbial growth in rumen has been proven by Seo *et al.* (2013); Uddin *et al.* (2015). An associative effect amongst the forages stimulates the microbial activity in rumen following addition of high nitrogenous supplements to poor quality roughages and hence, increases their intake and digestion (Niderkorn and Baumont, 2009). This associative effect might have induced a conducive environment for optimised rumen fermentation leading to higher nutrient intake as evident from the present study following novel feed supplementation.

Clinical chemistry

The haematological indices *viz*., Hb (gdL^{-1}) and haematocrit (%) were enhanced (P<0.01) in NFS than CON. The buffaloes fed NFS had higher (P<0.01) serum glucose (mgdl^{-1}) and lower (P<0.001) serum NEFA (ngml^{-1}) than buffaloes fed CON diet. Serum total protein, albumin, globulin, A:G ratio and total cholesterol were comparable (P>0.05) in NFS than CON groups. Serum urea (mgdl^{-1}) was significantly (P<0.05) increased in NFS than CON group (Table 3). Singh *et al.* (2013) reported significantly higher Hb and haematocrit concentrations with increasing dietary energy levels in lambs. Plasma glucose and NEFA are the principle key circulating blood metabolite to predict the energy status of the animals. NEB triggers adipose tissue breakdown of fat and results in an increased circulating NEFA concentration in dairy animals. Therefore, NEFA is considered as a marker for degree of under-nutrition and the severity of NEB and is characterized by low blood glucose, insulin, IGF-1 and high ketone bodies and NEFA concentrations (Sammad *et al*., 2022). In agreement with our results, dietary supplementation of concentrate mixture to cows has been reported to increase the plasma glucose concentration and decrease plasma NEFA and BHBA concentrations, thereby, improved energy balance (Kennedy *et al*., 2008; McNamara *et al*., 2003). Schöbitz *et al.* (2013) reported reduced plasma NEFA concentrations following dietary supplementation of concentrate mixtures in dairy cows as compared to their non-supplemented counterparts. Muralidharan *et al.* (2015) reported that supplementation of protein rich concentrate and urea molasses mineral block improved total protein and albumin in lambs. Cows supplemented with 2.5 kg extra concentrate per day had significantly higher total protein (Adjorlolo *et al*., 2019). Amanlou *et al.* (2017) also reported higher concentration of serum total protein and albumin in Holstein cows fed increasing dietary CP levels. BUN is considered as a useful metabolic indicator for evaluating the short-term or real-time changes in the N-utilization as well as nutritional profile of dairy animals (Islam *et al*., 2019). A positive correlation between higher concentration of blood urea in dairy animals fed increasing dietary CP levels has been reported (Amanlou *et al*., 2017). Dairy heifers fed protein rich concentrate diet showed significantly higher levels of serum urea and blood urea-N than non-supplemented heifers (Islam *et al*., 2019).

Serum minerals

Serum major minerals *viz.,* Ca and iP (mgdl^{-1}) were higher (P<0.01) in NFS than CON (Figure 2). Similarly, serum concentrations of trace minerals *viz*., Zn, Cu, Fe and Mn (µgml^{-1}) were also higher (P<0.01) in NFS than CON (Figure 3). Lambs supplemented with concentrate mixture exhibited improved concentrations of Ca and i-P (Muralidharan *et al*., 2015). Area specific mineral mixture has been reported to significantly improve the circulating concentrations of macro and trace minerals in normal as well as problematic dairy animals (Sahoo *et al*., 2017; Satapathy *et al*., 2016).

The enhanced level of serum minerals may be associated with the fact that novel feed supplement contained appreciable levels of minerals that might have increased the mineral status of the cows (Chaudhary *et al*., 2021).

Milk yield and composition

The daily milk yield (kg) was significantly (P<0.001) improved in NFS fed buffaloes (Figure 4). The total milk production (kg), 4% FCM, ECM, fat and protein yields (gd^{-1}) were also followed the similar trend. Milk composition showed no significant difference (P>0.05) between the groups (Table 4). The improved lactation performance of dairy animals 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). In agreement with the present results, several researchers have been reported an increased milk production, fat, protein, 4% FCM and ECM yields in dairy cows and goats fed concentrate mixtures (Adjorlolo *et al*., 2019; Mahfuz *et al*., 2018; McKay *et al*., 2019). Feeding of high energy corn silage increased the ruminal concentration of fermentable carbohydrates and made energy available for milk production, thereby, improved milk production, 4% FCM, ECM, fat and protein yields (Zhou *et al*., 2015).

Reproductive performance

Dietary supplementation of novel feed supplement to buffaloes considerably improved the conception rate (75%) as compared to CON (50%). The buffaloes fed NFS came into heat (oestrus) following 28.70 day, however, buffaloes in CON group become oestrus after 47.78 day and were inseminated. Following insemination, 33.33 and 16.67% of total animals from CON and NFS groups, respectively were returned to heat again and become pregnant in subsequent inseminations. A total of 25 and 8.33% of the animals were non-responsive and did not show any sign of oestrus in CON and NFS groups, respectively. The number of inseminations per conception was significantly (P<0.01) lower in NFS (1.22) than CON (2.1). The estradiol concentration ($pgml^{-1}$) remained comparable (P>0.05); however, progesterone concentration ($ngml^{-1}$) was significantly (P<0.05) higher in NFS relative to CON group (Table 5). Reproductive performance directly governs by plane of nutrition of animals and regulates the post-partum resumption of ovarian activities and subsequent events. Continuous supply of essential nutrients is required for gametogenesis and synthesis of blood metabolites related to ovulation and pregnancy (Robinson *et al*., 2006). Undernutrition or imbalanced nutrition predisposes to NEB, which impedes reproduction by inhibiting IGF-1 release, thereby preventing oocyte maturation before ovulation, reduces serum progesterone concentration and hence, fertility in dairy animals (Butler, 2005). In agreement with the present results, it has been reported that cows supplemented with extra concentrate had less proportion of non-cyclic animals (Adjorlolo *et al*., 2019). 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. Progesterone plays an important role in the reproductive events related to establishment and maintenance of pregnancy (Lonergan and Sánchez, 2020).

Metabolic hormones and total antioxidant capacity activity

The circulating concentrations of T_3, T_4, GH

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

Attributes	Concentrate mixtures		Wheat straw	Sugarcane top
	CON	NFS		
DM	90.45	90.62	90.56	28.24
OM	94.50	93.61	93.18	94.48
CP	15.48	25.53	3.53	5.95
EE	4.59	3.10	1.15	1.62
TA	5.50	6.39	6.82	5.52
NDF	38.86	41.24	81.53	69.95
ADF	14.19	13.23	53.90	43.76

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. Nutrient intake and body weight of buffaloes supplemented with novel feed supplement.

Attributes	Dietary treatments		P-value
	CON	NFS	
Body weights, kg			
0 d	407.28±11.25	409.6±10.25	0.880
60 d	410.09±11.49	415.03±12.35	0.773
120 d	412.63±11.30	423.91±11.14	0.485
Total gain (kg)	5.35±1.31^{b}	10.01±1.45^{a}	0.001
ADG (gd^{-1})	44.60±10.92^{b}	119.23±15.43^{a}	0.001
DM intake			
kgd^{-1}	9.87±0.27^{b}	11.64±0.21^{a}	0.000
gkg^{-1}W$^{0.75}$	108.70±3.37^{b}	127.15±3.67^{a}	0.001
% BW	2.40±0.05^{b}	2.79±0.06^{a}	0.005
Nutrient intake (gkg^{-1}W$^{0.75}$)			
CP	6.22±0.29^{b}	8.82±0.19^{a}	0.000
DCP	3.87±0.17^{b}	5.99±0.13^{a}	0.000
TDN	62.26±1.92^{b}	77.72±2.30^{a}	0.000
Nutrient density, %			
DCP	3.55±0.10^{b}	4.73±0.07^{a}	0.000
TDN	57.24±0.01^{b}	61.13±0.01^{a}	0.000

BW: Body weight, ADG: Avergae daily gain; DM: Dry matter; CP, Crude protein; DCP: Digestible crude protein; TDN: Total digestible nutrient. ^{ab}Mean±SE with different superscript in a row differ significantly.

Table 3. Haemato-biochemical profile of buffaloes supplemented with novel feed supplement.

Attributes	Dietary treatments		P-value
	CON	NFS	
Hb, gdl^{-1}	9.84 ± 0.29^{b}	12.87 ± 0.39^{a}	0.003
Hematocrit, %	25.71 ± 0.96^{b}	38.88 ± 1.03^{a}	0.000
Glucose, $mgdl^{-1}$	49.60 ± 1.08^{b}	63.70 ± 1.41^{a}	0.000
NEFA, $ngml^{-1}$	11.55 ± 0.96^{a}	6.83 ± 0.28^{b}	0.003
Total protein, gdl^{-1}	6.55±0.17	6.86±0.15	0.219
Albumin, gdl^{-1}	3.27±0.11	3.49±0.07	0.090
Globulin, gdl^{-1}	3.28±0.20	3.37±0.15	0.726
A:G ratio	1.02±0.10	1.06±0.06	0.718
Urea, $mgdl^{-1}$	25.95 ± 1.75^{b}	31.95 ± 1.10^{a}	0.016
Total cholesterol, $mgdl^{-1}$	97.44±5.53	96.08±5.52	0.879

Hb: Hemoglobin; NEFA: Non-Esterified Fatty Acid; A:G: Albumin: Globulin. ^{ab}Mean±SE with different superscript in a row differ significantly.

Table 4. Milk yield and composition of buffaloes supplemented with novel feed supplement.

Attributes	Dietary treatments		P-value
	CON	NFS	
Daily milk yield, kgd^{-1}	3.23 ± 0.17^{b}	4.45 ± 0.17^{a}	0.000
Total milk yield, kg	387.60 ± 20.10^{b}	533.54 ± 19.83^{a}	0.000
4 % FCM, kgd^{-1}	4.71 ± 0.35^{b}	6.63 ± 0.43^{a}	0.002
ECM, kgd^{-1}	4.80 ± 0.37^{b}	6.81 ± 0.35^{a}	0.001
Fat yield, gd^{-1}	227.12 ± 16.97^{b}	323.68 ± 17.95^{a}	0.001
Protein yield, gd^{-1}	103.30 ± 10.06^{b}	140.50 ± 9.15^{a}	0.012
Milk composition, %			
Fat	7.05±0.51	7.28±0.62	0.768
Protein	3.04±0.07	3.16±0.09	0.287
Lactose	4.47±0.12	4.75±0.13	0.114
SNF	8.18±0.16	8.68±0.21	0.171
Total solid	15.23±0.53	15.96±0.79	0.445
Ash	0.71±0.01	0.72±0.01	0.686

FCM: Fat Corrected Milk; ECM: Energy Corrected Milk. ^{abc}Mean±SE with different superscript in a row differ significantly.

Table 5. Reproductive performance of buffaloes supplemented with novel feed supplement.

Attributes	Dietary treatments		P-value
	CON	NFS	
Total no. of animals selected	12	12	-
Total No. of animals confirmed pregnant	6	9	-
Conceived at first insemination	2	7	-
Conceived at subsequent insemination	4	2	-
Non-responsive	3	1	-
First oestrus after start of feeding (days)	47.78± 5.11[a]	28.70±2.63[b]	0.003
No. of inseminations per conception	2.10±0.18[a]	1.22±0.15[b]	0.002
Conception rate (%)	50.0	75.0	-
Estradiol, $pgml^{-1}$	846.26±252.39	528.43±125.57	0.292
Progesterone, $ngml^{-1}$	4.44±1.81[b]	12.65±1.05[a]	0.004

[ab]Mean±SE with different superscript in a row differ significantly.

Table 6. Metabolic hormone profile and total antioxidant capacity assay of buffaloes fed novel feed supplement.

Attributes	Dietary treatments		P-value
	CON	NFS	
T_3, $ngdl^{-1}$	2.17±0.15[b]	3.31±0.20[a]	0.002
T_4, $ngdl^{-1}$	60.72±1.83[b]	71.18±2.30[a]	0.008
GH, $ngml^{-1}$	2.83±0.31[b]	4.52±0.48[a]	0.018
IGF-1, $ngml^{-1}$	22.73±1.26[b]	38.82±2.19[a]	0.000
TAC, UmL^{-1}	1.82±0.31[b]	4.69±0.33[a]	0.000

T_3: Triiodothyronine; T_4: Thyroxine; GH: Growth hormone; IGF-1: Insulin-like growth factor-1; TAC: Total antioxidant capacity. [ab]Mean±SE with different superscript in a row differ significantly.

Table 7. Cost: benefit ratio analysis of NFS supplementation in buffaloes.

Attributes	Treatment groups	
	CON	NFS
Feed cost, Rs/d		
Concentrate	30.96	48.87
Roughages	45.91	52.08
Green	20	20
Total	96.87	120.95
Feed cost/ lit milk	29.81	27.12
Value of milk, Rs/d	162.35	223.23
Net revenue, Rs/d	65.48	102.28
Additional feed cost, Rs/d	-	24.08
Net benefit, Rs/d	-	36.80
BCR*	1.68	1.85

BCR, Benefit-cost ratio; Wheat straw, Rs 6/kg; Green, 4/kg; CON (concentrate mixture), 18.30/kg; NFS (novel feed supplement), 23.60/kg, Sale price of milk, Rs 50/L.

and IGF-1 (ngml^{-1}) hormones were higher ($P<0.05$) in NFS than CON (Table 6). The concentrations of thyroid hormones are directly affected by level of feeding and reduces with lower feed intake in animals. In agreement with our results, a significantly higher circulating concentration of thyroid hormones following dietary energy supplementation to lactation dairy cows has been reported (Kirovski *et al*., 2012), which establish a positive correlation with energy balance in dairy cows during early lactation. The significant difference in serum IGF-1 concentration in NFS group in our study agrees with previous reports of Moriel *et al.* (2012) demonstrating nutrient supplementation was associated with increased concentrations of plasma IGF-1 in underfed cows. The dietary supplementation of concentrate mixture or energy rich diet has been reported to improve the plasma concentration of IGF-1 in cows (Grala *et al*., 2011; Kirovski *et al*., 2012). Dietary protein supplementation has also been reported to increase the circulating concentrations of IGF-1 in cows (McLean *et al*., 2018). NEB down regulates the GH receptors in liver leading to uncoupling of GH-IGF axis, consequently, reduction in the circulating IGF-1 concentration despite the increase in GH (Lucy *et al*., 2009). Therefore, the increased concentration of serum IGF-1 in the present study following dietary supplementation of NFS supplement to dairy cows is an indicative of a GH-responsive liver and hence a recoupled somato-tropic axis (Lucy *et al*., 2009) which further revelled that the animals are recovering from NEB.

The TAC activity (UmL^{-1}) was also found to be significantly ($P<0.001$) higher in NFS than CON group (Table 6). NEB increases the concentrations of ketone bodies and NEFA (Sammad *et al*., 2022) and oxidation of these metabolites results in an increased production of reactive oxygen species thereby oxidative stress in cows. Pedernera *et al.* (2010) reported that early-lactating dairy cows fed restricted production ration had lower

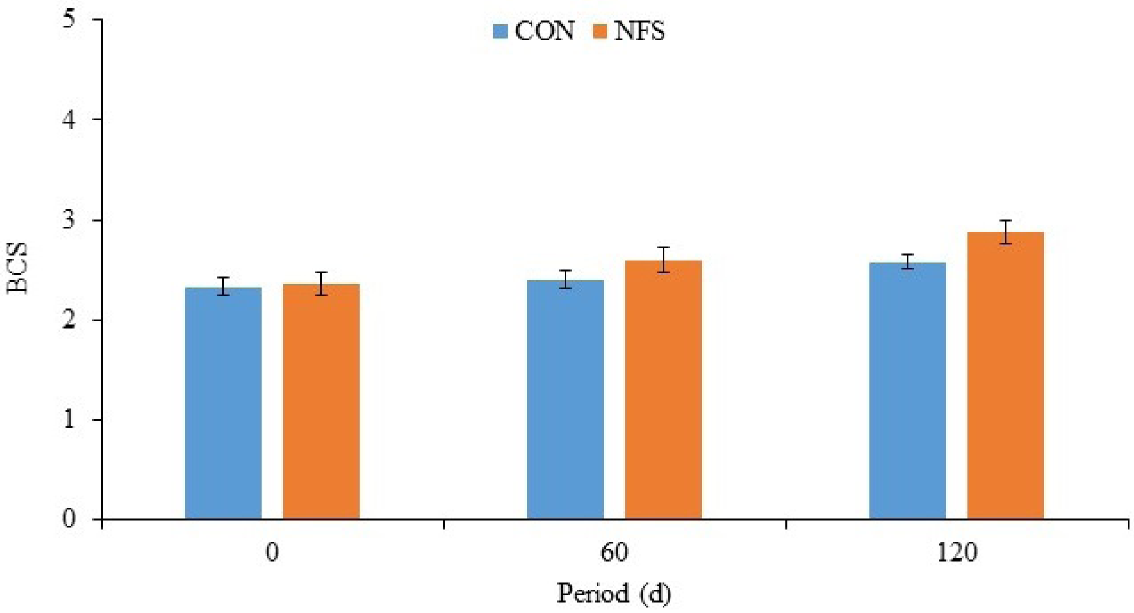

Figure 1. BCS of buffaloes supplemented with novel feed supplement.

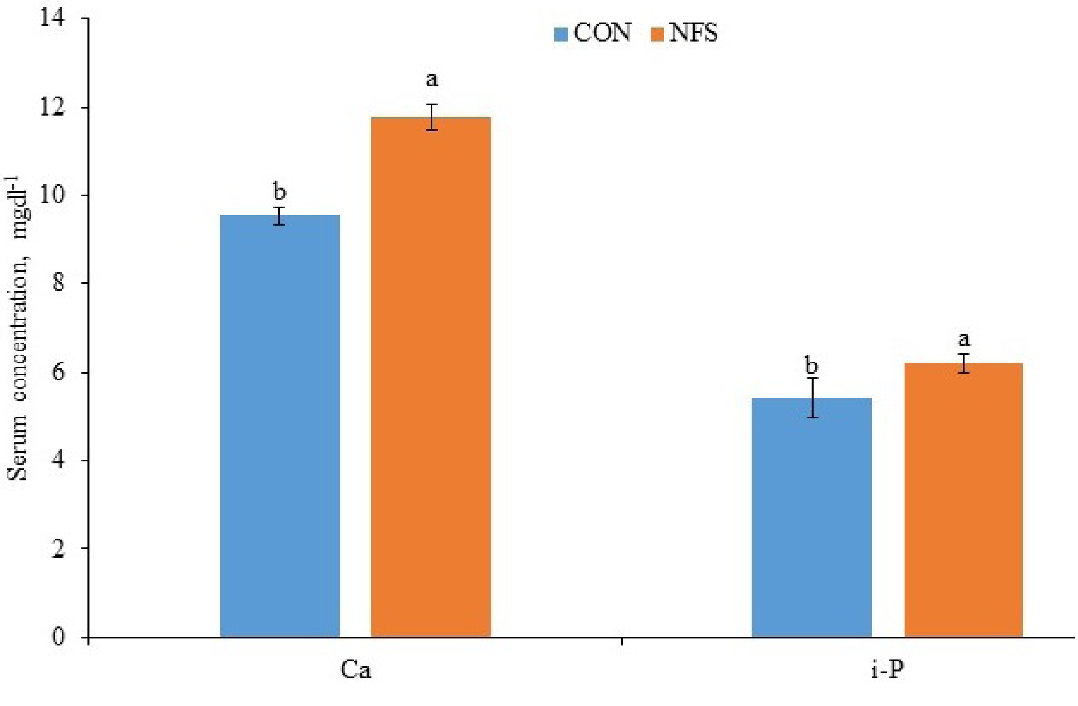

Figure 2. Serum macro mineral concentration of buffaloes supplemented with novel feed supplement.

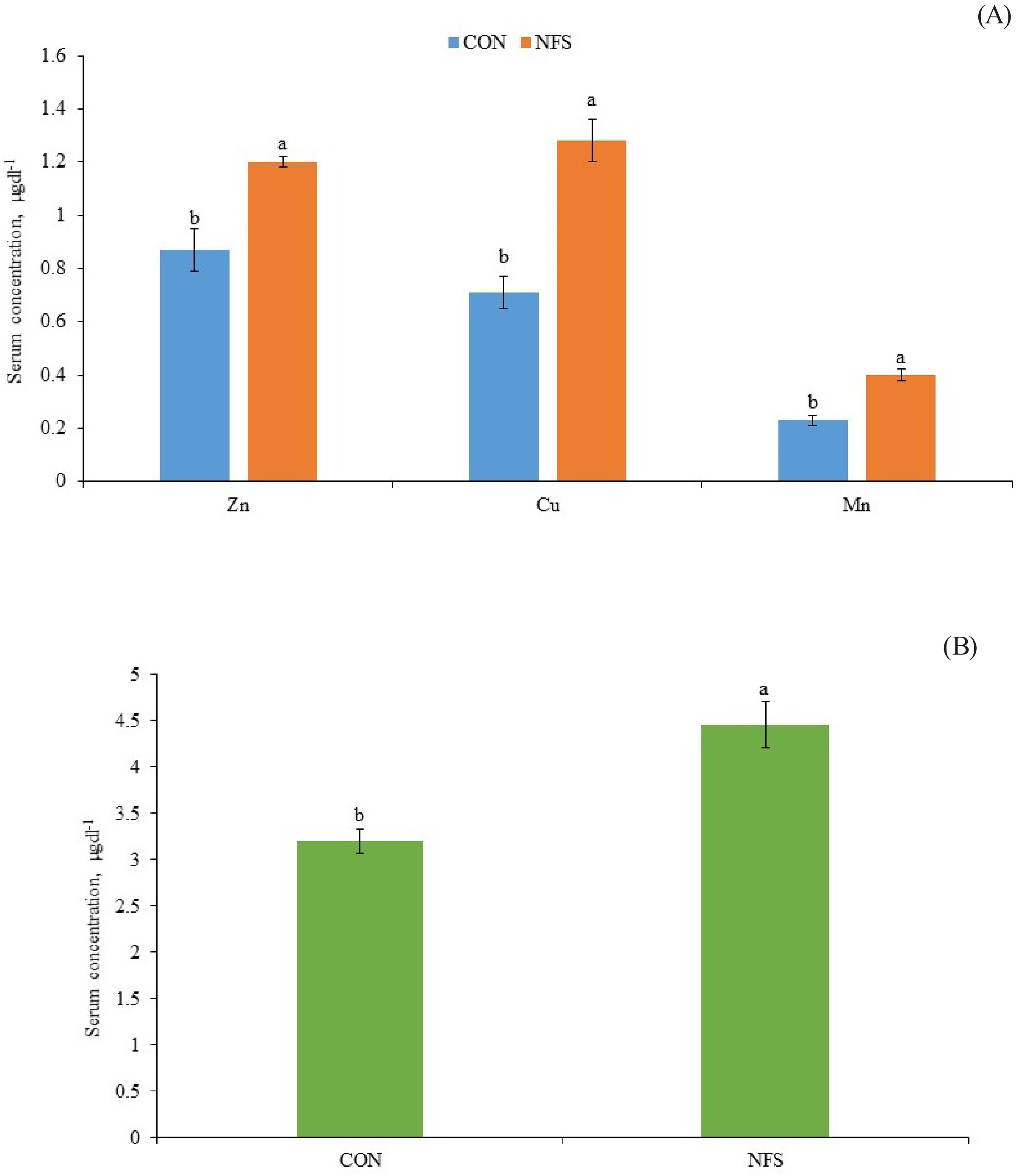

Figure 3. Serum trace mineral concentration of buffaloes supplemented with novel feed supplement: (A) Zn, Cu and Mn, (B) Fe.

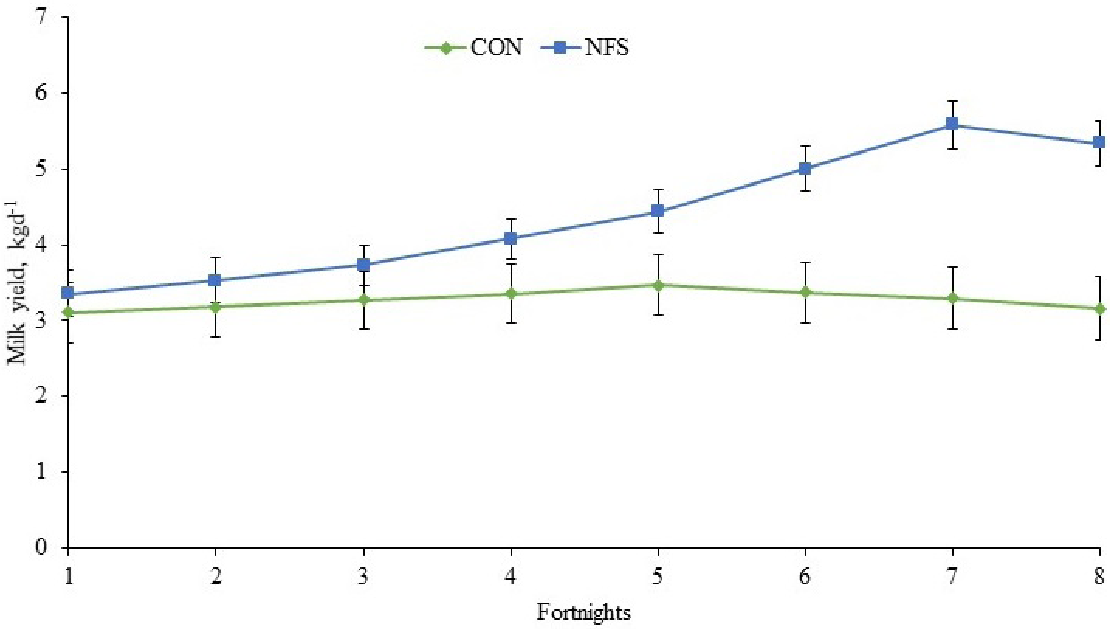

Figure 4. Daily milk yield of buffaloes supplemented with novel feed supplement.

concentration of biological antioxidant potential and higher oxidative stress as compared to the cows fed high production ration.

Socio economic implications

Inclusion of novel feed supplement to straw-based diet considerably improved the lactation performance of buffaloes owing to an improved intake and nutrients availability as well as associative effects among the dietary constituents. The analyses of cost benefit of milk yield over 120 days of lactation period revelled that the strategic supplementation of NFS enhanced the overall cost of feeding, however, surplus returns via increased milk yield not only compensated the extra cost but also yielded higher net revenue (Rs/d) in NFS fed buffaloes (102.28) than CON diet fed buffaloes (65.48). The net benefit (Rs/d) was 36.80 in NFS supplemented group. A benefit-cost ratio (BCR) is an indicator showing the relationship between the relative costs and benefits of experiment, expressed in monetary or qualitative terms. The BCR was also higher in NFS than CON (Table 7).

Based on above results, it can be concluded that strategic supplementation of the novel feed supplement (0.25% of body weight) significantly improved the nutrient intake, energy balance, metabolic profile, milk yield and reproduction performance of problematic buffaloes under field condition during early post-partum period.

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