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Specific supplemental feeding of Sahiwal cows and their effect on body condition score, body weight and onset of reproductive cycle

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Abstract
Focusing on the state of negative energy balance in cows during transition period the present research was designed. Sixteen advanced pregnant Sahiwal cows were selected and divided into two groups *i.e.,* control (n=8) and treatment (n=8). The cows in control group were fed with available green fodder along with dry straw, concentrate @ 3 kg/day/animal, lactating cows were provided with 1 kg concentrate for maintenance along with 400 gm concentrate/litre milk/day and mineral mixture was given @ 2% of total feed/animal/day. The cows in treatment group were fed with additional experimental feed *i.e.,* Bypass fat (100 g/animal/day) + Bypass protein (200 g/animal/day) + Beta-carotene (300 mg/animal/day), for a period of 70±10 days. The Body Condition Score (BCS), Body weight (BW) and first post-partum estrus were recorded. Results showed that BCS was 2.91±0.07, 2.87±0.08 and 2.81±0.09, 3.19±0.06 and BW (in kgs) was 337.93±3.82, 297.12±8.28 and 338.31±2.17, 322.68±5.25 in control and treatment group recorded at -10 and 60th day post-partum respectively. The first post-partum estrus in treated group differs significantly ($p<0.05$) *i.e.,* 44.13 ± 1.09 and 39.25 ± 0.37 days in control and treatment group respectively. So, it is concluded that BCS and BW was improved as the losses observed in both the parameter was less in treatment group as compared to control group. Also, the first detectable post-partum estrus was found earlier in treatment group over control group. Hence, feeding bypass nutrients in the diet of indigenous dairy cows improved their growth as well as reproductive performance.

Keywords: Sahiwal, Body Condition Score, Body Weight, Estrus, Bypass nutrients

Introduction
Nutrient requirement of dairy animals under Indian conditions are being recently refined for precise feeding of animals to get better stimulus in production and reproduction performance in economically efficient way (Sarkar *et al*., 2022) [25]. The negative energy balance in early lactation causes delayed post-partum ovarian activity (Garnsworthy and Webb 1999) [6], apart from affecting peak milk yield and overall lactation yield. The level of non-esterified fatty acids (NEFA) increases in plasma as a consequence of body fat mobilization (Reid and Collins, 1980) [22] and leads to hepatic lipidosis. In ration of early lactating or high yielding dairy animals, fat is helpful in increasing the energy density of the diet which is 2.25 times more than carbohydrate. The supplementation of fat in animals' ration checks negative energy balance during early lactation, acidosis and laminitis and integrate fatty acid into milk fat. Peak milk yield occurs 6 to 8 weeks postpartum, while maximum feed intake lags behind peak milk yield by several weeks (Sharma *et al*., 2021) [28].
Considering above facts, transition nutrition has gained an important significance among researchers worldwide due to various metabolic disorders associated during this phase which determines the subsequent productive and economic losses (Remppis *et al*., 2011) [23]. During transition, dairy animals require better energy supplements to minimize deleterious effects caused by reduced dietary intake and body weight losses (Katiyar *et al*., 2019) [11]. Furthermore, extensive degradation of high-quality nutrients in rumen often renders their availability to the host and leads to wastage of nutrients. Therefore, in order to provide quality and precise nutrition to dairy animals during periods of high nutrient demand, use of protected/bypass dietary nutrients can be a promising strategy for enhancing both the quality

and quantity of animal production. Various methods such as heat treatment, chemical treatment, encapsulation, selective manipulation of rumen metabolic pathways have been employed to protect or decrease degradation of nutrients in the rumen (Ganai *et al*., 2019) [4]. In tropics, majority of the diets are dominated by crop residues, low in energy, protein, and minerals (Qureshi *et al*., 2002) [18]. Therefore, feeding low energy and protein diet to lactating animals leads to negative energy balance (NEB), thereby decreasing productive and reproductive performance (Ranjan *et al*., 2012) [20].

The objective of the present work was to investigate the effect of Bypass nutrients (fat and protein) along with beta carotene as energy, protein and antioxidant agent respectively in combination, to deal with two major peripartum issues *i.e.*, Negative energy balance (NEB) and Oxidative stress, which impacts significantly on the resumption of next reproductive cyclicity in cows.

Materials and Methods

The present study was conducted at Instructional Dairy Farm (IDF), of G. B. Pant University of Agriculture and Technology, Pant Nagar, from October, 2023 to April 2024. The main campus of university lies in Udham Singh Nagar district of Uttarakhand at 29° N latitude and 79° E longitude at an elevation of 243.8 m above the mean sea level.

Sixteen (16) advanced pregnant Sahiwal cows were selected randomly at IDF, and were further divided into two groups *i.e.*, control (n=8) and treatment (n=8). The selected animals were maintained on iso-managerial conditions and fed as per Table 1. The animals under treatment group were fed with additional experimental diet along with standard feeding, experimental diet was initiated -15 to -10 days before expected day of calving and continued till 60th day post-partum. The experimental diet contains Bypass fat (100 g/animal/day) + Bypass protein (200 g/animal/day) + Beta-carotene (300 mg/animal/day). The additional 10 g bypass fat/litre of milk produced was added until 60 days post-partum (Gowda *et al*., 2014) [7] taking care that it should not exceed 250 g/day/animal (Nirwan *et al*., 2021) [15].

Feeding of both the groups were as follows:

Table 1: Feeding Schedule of both the groups under study.

Control group (n=8)	Treatment group (T) (n=8)
Available Green fodder + Dry straw Concentrate @ 3 kg/day/animal -Lactating: 1 kg maintenance + 400 gm/litre milk/day Mineral mixture @ 2% of total feed/animal/day	Available Green fodder + Dry straw Concentrate @ 3 kg/day/animal -Lactating: 1 kg maintenance + 400 gm/litre milk/day Mineral mixture @ 2% of total feed/animal/day Bypass fat @ 100 gm/day/animal + 10 g/l of milk Bypass protein @ 200 gm/day/animal Beta-carotene @ 300 mg/animal/day

Sample collection: The blood samples were collected in vacutainer from jugular vein using 18G needle and the serum was separated within 30 minutes of collection by centrifugation at 3000rpm for 10 minutes. The serum obtained was marked for identification and stored at (-) 20 °C until analysis in 2 ml Eppendorf tubes. The collection of blood sample was done at 10 days interval from -10, 0 (*i.e.*, the day of calving), 10, 20, 30, 40, 50 and 60th day post-partum.

BCS was recorded before and after feeding trial *i.e.*, on 10-15 days before expected date of calving and on 60th day post-partum. The body condition score of the animal was evaluated as per Paul *et al*. (2020) [16]. As routine management the post-partum cows were observed twice a day for the occurrence of estrus, based on vaginal discharges and behavioral signs such as cows stand to be mounted by other cows, reduction in feed and water consumption; attempt to mount other cows, mucus discharge, sniffing the genitalia of other cows, swelling and reddening of the vulva, chin resting and back rubbing of another cow and increased vocalization (Röttgen *et al*., 2018) [24]. Duration from calving to the first post-partum estrus was recorded in days (Nirwan *et al*., 2019; Yoshida and Nakoa, 2005) [15, 35]. The BW of the animal was recorded before and after the end of experiment. For calculation of BW (Wangchuk *et al*., 2018) [32] Schaeffer's formula was found most reliable ($W = (L \times G^2)/300$, where, W, L and G signifies body weight (in lbs), Length (in inches) and Chest Girth (in inches)). The length is measured from point of shoulder to pin bone. The final calculated weight was in pounds and was converted in kilogram.

Statistical Analysis

All data collected was analyzed using software SPSS. Data represented as Mean±SE and considered significant at $p<0.05$.

Results and Discussion

The experimental diet was fed to the treated animal for approximately 70±10 days in total and its effect on various parameters was monitored.

Body Condition Score: Present feeding trial on advanced pregnant Sahiwal dairy cows showed the improvement in BCS on group fed with experimental diet after 60 days of feeding. The BCS recorded before starting of trial and on day 60 post-partum was 2.91±0.07, 2.87±0.08 and 2.81±0.09, 3.19±0.06 in control and treatment groups respectively. There was reduction in BCS, observed in both the groups before and after treatment but the loss in treatment group was lesser as compared to control group (Table 2). This indicates that the experimental diet had positive effect on BCS of selected Sahiwal cows. The BCS around calving, along with subsequent changes such as BCS at calving, and the speed and extent of BCS decline post-calving, can serve as indicators for elevated levels of non-esterified fatty acids, potential postpartum diseases, and variations in production performance (Rathbun *et al*., 2017) [21].

Table 2: Body Condition Score recorded before and after experimental period in control and treatment group.

Days	Control	Treatment
-10	$2.91±0.07^{aA}$	$2.87±0.08^{aA}$
60	$2.81±0.09^{aB}$	$3.19±0.06^{bB}$

Figures with different superscripts (A, B) within a column differ significantly (p<0.05)
Figures with different superscripts (a, b) within a row differ significantly (p<0.05)

Wildman *et al.* (1982) [33] stated that the BCS is an interpretive measure of body tissue reserves that are generally used to monitor energy balance. This can be considered effective indicators for health, reproductive performance and positive metabolic equilibrium of cattle. BCS of 3.5 is considered most optimum for reproduction and production performance in heifers and adult cattle. Elevated BCS greater than 4 up to 5 are obese and are associated with delayed conception, abortion and extending the days for initiation of lactation (Cargile and Tracy, 2021) [2].

Our results are in accordance with Mahajan *et al.* (2022) [12] who recorded that the feeding of the experimental diet resulted in significant improvement in the BCS of the heifers supplemented with bypass fat and bypass protein. The significant difference was also observed by Sharma *et al.* (2016) [27], supplemented prilled bypass fat @ 100 g/day pre-partum and 150 g/day in Murrah buffaloes during post-partum and observed the improvement in BCS in supplemented group over control group. Garg and Mehta (1998) [5], observed that the BCS of the cows improved due to bypass fat feeding indicating reduction in weight loss in the first quarter and helped gaining substantially after 90 days of feeding.

Similarly, the types of protein sources which resist degradation by the ruminal microbes and reach the abomasum basically unaltered are termed as 'protected protein' or bypass protein (Merchen and Titgemeyer, 1992) [13] and their supplementation in dairy cattle ration has resulted in significant improvement in body condition (Anderson *et al.*, 2001) [1]. However, Kolver and Muller (1998) [12] have documented no significant changes in BCS of cows fed with bypass protein.

Body weight: The recorded body weight (in kgs) before and after experimental diet supplementation was 337.93±3.82, 297.12±8.28 and 338.31±2.17, 322.68±5.25 kg in control and treatment groups respectively (Table 3). The body weight of the selected animals differs significantly within the groups before and after the end of experiment and there was significant (p<0.05) difference between group also on 60^{th} day post-partum. The loss in body weight was observed 60 days post-partum in both the groups but it was more in control group as compared to treatment group.

Table 3: Body Weight (kgs) before and after experimental period.

Days	Control	Treatment
-10	$337.93±3.82^{Aa}$	$338.31±2.17^{Aa}$
60	$297.12±8.28^{Ba}$	$322.68±5.25^{Bb}$

Figures with different superscripts (A, B) within a column differ significantly (p<0.05)
Figures with different superscripts (a, b) within a row differ significantly (p<0.05)

Our findings are supported by many authors as Mishra *et al.* (2022) [14] reported that buffaloes in the control group experienced greater body weight loss over the experimental period, whereas those fed the bypass fat diet lost less weight and exhibited better production. Vahora *et al.* (2013) [30] observed that the loss in BW was prevented in lactating buffaloes supplemented with bypass fat group as compared to non-supplemented group. Singh *et al.* (2014) [29] observed at the start of the experiment, the metabolic body weight was higher (p<0.05) in the control cows. However, after 90 days, cows supplemented with protected fat showed a significant increase in metabolic weight (p<0.05).

Also, Grewal *et al.* (2014) [9] observed more body weight gain by supplementation of bypass fat and niacin in cows. Gowda *et al.* (2013) [8] reported less body weight loss in cows fed protected fat and the regain of body weight was much quicker, also Yadav *et al.* (2015) [34] reported less body loss due to feeding prilled fat to crossbred cow. The postpartum body weight of prilled fat of supplemented group @ 100 g/day/buffalo was significantly (p<0.05) more and the decline in BW on day of parturition was less in comparison to control group (Sharma *et al.*, 2016) [27]. The possible cause behind the minimal weight loss observed in Sahiwal cattle in the treatment group was due to the addition of bypass fat and protein, which led to decreased catabolic activity and reduced use of body reserves.

On contrary Wadhwa *et al.* (2012) [31] who reported that the BW of the animals improved considerably in the bypass fat supplemented group as compared to the control group (551 vs 508, kg), but the differences were non-significant.

First post-partum estrus: Occurrence of first post-partum detectable estrus was observed on 44.13 ± 1.09 and 39.25 ± 0.37 days in control and treatment group respectively (Table 4). It is noticeable that after supplementation of bypass fat and protein along with beta-carotene, results in the occurrence of first post-partum estrus preponed by approximately 5 days as compared to control group animals.

Table 4: 1^{st} post-partum detectable estrus in both the groups.

Groups	1^{st} post-partum estrus (days)
Control	44.13 ± 1.09
Treatment	39.25 ± 0.37*

* differ significantly (p<0.05) between groups.

The commencement of cyclicity was related with the process of involution of uterus, as the duration for uterine involution was reduced on bypass protein and fat supplementation, which may be responsible for relatively early onset of heat. Our findings are in accordance with Nirwan *et al.* (2019) [15], who observed the mean duration for exhibiting first post-partum estrus were 35.2±1.25 and 33.13±0.53 days by control and treatment group respectively. Similar results were also observed by Savsani *et al.* (2013) [26] and Ramteke *et al.* (2014) [19] that showed a shorter period for the occurrence of first postpartum heat in the bypass fat supplemented group in buffaloes. In bypass fat supplemented buffaloes, Prajapati (2018) [17] found that postpartum heat occurred 14 days earlier (p<0.05) than in the control group. In contrary to our findings, Katiyar *et al.*, (2017) [10] showed non-significant (p>0.05) difference observed in days to first overt estrus among the study groups (control: 37.88±6.57, T_1 (bypass fat): 42.33±12.94, T_2

(bypass protein): 29.37±5.35, T_3 (bypass fat + bypass protein): 30.6±6.73).

Conclusion
Hence, it is concluded that feeding bypass nutrients and beta-carotene in combination can be helpful to deal with NEB as well as oxidative stress, during peri-partum period, as in present study the BCS and BW improved following supplementation of bypass nutrients and days to occurrence of first postpartum estrus was also reduced which is indicative of positive effect of Bypass nutrients on uterine involution.

Conflict of interest
The Authors declares no conflict of interest.

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