Archives of Current Research International

Volume 25, Issue 7, Page 667-675, 2025; Article no.ACRI.140570
ISSN: 2454-7077

Impact of Adding Poly-herbal Preparations and Mustard Oil Supplements on the Reproductive and Production Performance of Sahiwal Cows

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Authors' contributions

This work was carried out in collaboration among all authors. Author SC designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Author CK managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/acri/2025/v25i71368

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/140570

Original Research Article

Received: 07/05/2025
Accepted: 15/07/2025
Published: 18/07/2025

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Cite as: *Chandra, Subhash, Chandan Kumar, and P S Oberoi. 2025. "Impact of Adding Poly-Herbal Preparations and Mustard Oil Supplements on the Reproductive and Production Performance of Sahiwal Cows". Archives of Current Research International 25 (7):667-75. https://doi.org/10.9734/acri/2025/v25i71368.*

ABSTRACT

The present experiment was conducted to evaluate the effect of supplementation with mustard oil and poly-herbal preparations on the productive and reproductive performance of Sahiwal cows. For this study, 40 Sahiwal cows were selected 30 days before calving, and these Sahiwal cows were randomly divided into four groups (control T0 group and supplemented T1, T2 and T3 groups), with 10 animals in each group. Group T1 was supplemented with mustard oil, group T2 was supplemented with a poly-herbal preparations, and group T3 was supplemented with both mustard oil and the poly-herbal preparations. However, group T1 (n = 10) received an additional 200 ml of mustard oil from 21 days before calving to 21 days after calving, group T2 (n = 10) received an additional poly-herbal preparation (150 g herbal mixture, 25 g kalanamak, and 250 g jaggery) and fed it to the cow up to seven days from the date of calving, and group T3 (n = 10) received an additional mustard oil as T1 group and an additional poly-herbal mixture as T2 group. The poly-herbal mixture was prepared by mixing six herbs (Methi, Ajwain, Sanuf, Sowa, Sundh and Bari Elaichi), as well as jaggery and kalanamak (black salt). Animals in the supplemented and control groups received rations as per the NRC's (2001) feeding schedule. The results depicted lower incidences of RFM (retention of fetal membrane) in the supplemented groups, *i.e.,* T1 (20%), T2 (10%), and T3 (10%), as compared to the control group (30%). The time required for expulsion of the placenta was significantly (P<0.05) lower in the supplemented (T1, T2, and T3) groups as compared to the control (T0) group. The average days to first insemination/AI were significantly (P <0.05) lower in the supplemented groups as compared to the control group. The service period in Sahiwal cows supplemented with a poly-herbal mixture and mustard oil (T1, T2, and T3) was significantly lower (P <0.05) than in the control group. There was an improvement in conception rate in T1 (19%), T2 (12%), and T3 (24%), compared to the T0 group. The incidence of metritis was lower in the supplemented groups T1(20%), T2(10%) and T3(10%) as compared to the control group (T0-30%). Milk yield recorded up to 12 weeks was significantly (P<0.05) higher in T2 and T3 groups as compared to the control group (T0). The mean values of BHBA and NEFA concentrations were significantly (P<0.05) lower in T1 and T3 as compared to the T0 respectively. Supplementation of mustard oil and a poly-herbal mixture safely included in the diet of peri-parturient animals subsequently helps in improving the reproductive efficiency and productive performances of Sahiwal cows.

Keywords: Mustard oil; poly-herbal mixture; transition period; productive and reproductive performance; Sahiwal cows.

1. INTRODUCTION

India has emerged as a world leader in milk production and currently has a production capacity of 239.30 million tons per year (BAHS, 2023-24). Sahiwal is one of the important milch breeds of cattle in the Indian subcontinent, with remarkable powers of heat tolerance, disease resistance, low maintenance costs, being less susceptible to food poisoning, and being resistant to both external and internal parasites. High levels of milk produced by today's dairy cows create a challenge in meeting the animals' energy requirements during early lactation. The resulting negative energy balance impairs reproduction. The transition period is characterized by a substantial decline in feed intake and a negative energy and protein balance. Negative energy balance during transition had an adverse effect on the fertility of animals (Nebel and Gilliard, 1993). The period around parturition in dairy cattle is characterized by negative energy balance, reduced feed intake, and an increase in NEFA and BHBA. Cattle are exposed to an increased risk of infection of the uterus as the anatomical barriers are breached and genital organs remain open for several days during parturition. It is estimated that around 18–40% of cattle are culled per year due to infertility or sterility in India (Kaikini, 2002). Post-partum fertility has a profound impact on the economic viability of the dairy industry. Among various factors, periparturient disorders have been recognized as the most important factors influencing fertility. Among parturition-associated problems (like dystocia, stillbirth, abortions, retained placenta, etc.), RFM and dystocia have been recognized as the most important factors compromising the future reproductive life of the animal (Noakes et al., 2009). Nutritional supplements during this period may therefore provide some support to overcome the adverse effects of impaired metabolic and immune functions (Dai et al., 2011). Energy and protein

are the two most significant factors affecting milk performance. Incorporating lipids into rations for dairy cattle usually increases the energy density of the ration and improves lactation and reproductive performance. Mustard oil is traditionally used in the feeding of Indian dairy cows, which contains 60% monounsaturated fatty acids (MUFA) and 40% polyunsaturated fatty acids (PUFA). Also, mustard oil has a favorable effect on milk composition (Dhiman et al., 2000; Zheng et al., 2005), because it contains high levels of unsaturated fatty acids. Herbal preparations relieve the heat stress in dairy cows, effectively restore the altered milk constituents, and increase milk production in cows with sub-clinical mastitis (Zhang et al., 2007). Poly-herbal formulations containing balanced phytoestrogens have been used to induce estrus and improve overall breeding efficiency in dairy animals (Nemade et al., 1994).

2. MATERIALS AND METHODS

The present study was conducted on Sahiwal cows in the transition phase maintained at the livestock research center (LRC) of the National Dairy Research Institute (N.D.R.I.), Karnal. It is located at 290 43' N latitude and 760 58' E longitudes at an altitude of 245 meters above the mean sea level in the bed of the Indo-Gangetic alluvial plain. The present study was conducted from the last week of August 2014 to the last week of March 2015 on 40 advanced pregnant Sahiwal cows who were selected 21 days before the expected date of calving and randomly allotted on the basis of their body weight, parity, and age to one control (T0) and three different supplemented (T1, T2 and T3) groups of 10 cows each, under a randomized complete block design (RCBD). The control group, i.e., T0 (n = 10), was offered as the standard requirement for the NRC (2001). Three different supplemented groups (T1, T2 and T3) were offered rations as per the control group and different supplements. In T1 group, cows were supplemented with mustard oil (M) (200 g/animal/day) 21 days before calving to 21 days after calving. In T2 group, cows were supplemented with a poly-herbal mixture preparation including Trigonella foenum-*graecum* (Methi) 25 g, *Trachyspermum ammi* (Ajwain) 25 g, *Foeniculum vulgare* (Saunf) 25 g, *Anethum graveolens* (Sowa) 25 g, *Zingiber officinale* (Sundh) 25 g, *Elettaria cardamomum* (Bari elaichi) 25 g, Kala namak 25 g, and 250 g Jaggery. The polyherbal mixture was prepared by mixing each herb ingredient, making a total quantity of 150 g, and 25 g of kalanamak was mixed in 1 liter of water, and the mixture was boiled for 20–30 minutes till the volume of water became half. Subsequently, the jaggery was added and further boiled for another 5–6 minutes. The above-prepared poly-herbal mixture was mixed with the concentrate mixture and fed to the cow for seven days postpartum from the date of calving. In T3 group, cows were supplemented with mustard oil (as in the T1 group) and poly-herbal mixture preparation (as in the T2 group). Reproductive parameters of cows were recorded, which include incidence of retention of fetal membranes (%), time required for expulsion of fetal membranes, days to first insemination (days), days open (days), conception rate (%), and incidence of metritis (%). Hand milking was done twice a day at 4.30 AM and 4.30 PM, and the milk yield of individual animals was recorded up to 12 weeks. To determine the milk composition, 50 ml of milk samples from individual Sahiwal cows were collected at a weekly interval in properly cleaned milk sample bottles. Milk samples were analyzed by using lactostar automatic milk analyzer (Funke Gerber, model no. 3510-055007) for milk fat, protein, lactose, SNF, and total solids on 7th, 14th and 21stdays after the calving. Blood samples were collected in 10 ml heparinized (20 IU heparin/ml blood) tube at 7:30 a.m. before offering any feed from the jugular vein on the days of -21, -15, 0, +14, and +21 before and after calving from all animals. The plasma was harvested within one hour after sampling, followed by centrifugation at 2500 RPM for 30 minutes at 4°c and plasma was collected and stored at -20°c in the storage vials of 2 ml capacity till the analysis of the plasma for beta-hydroxybutyrate (BHBA) and non-esterified fatty acids (NEFA). The amount of plasma beta-hydroxybutyrate (BHBA) was calculated using an ELISA kit from Cayman Chemical Company, Ann Arbour, USA. By using a competitive inhibition enzyme immunoassay approach, the bovine NEFA kit calculated the plasma non-esterified fatty acid (NEFA). Every estimate was made in accordance with the guidelines provided by the manufacturers. All data were subjected to ANOVA for RCBD using the general linear model (GLM) of SAS (SAS Institute, 2002), and group comparison was done by the turkey test.

3. RESULTS AND DISCUSSION

Results showed the significance of mustard oil (before and after one month postpartum) and poly-herbal mixture (just after postpartum) feeding during the transition phase to mitigate

the parturition stress and facilitate early expulsion of the fetal membrane. Table 1 shows the impact of feeding mustard oil, a poly-herbal mixture, and their combination on factors associated with the reproductive and productive performance of Sahiwal cows. The study's findings demonstrated that, there were lower incidences of RFM in the supplemented groups, i.e., T1 (20%), T2 (10%), and T3 (10%), as compared to the control group (30%).These findings demonstrate that the addition of mustard oil to the diet of Sahiwal cows reduced the incidence of RFM and the associated postpartum issues, most likely by improving nutritional status and reducing periparturient stress in the animals. Mariano et al. (2004) state that animals that retain their fetal membrane are more likely to develop metritis or endometritis, have lower fertility, and experience slower uterine involution. Significantly less time was taken to expel the placenta in the supplemented groups T1 (4.948±0.21h), T2 (5.847±0.16h), and T3 (9.406±0.22h) than T0 (9.406±0.22h). Vaghasiya, (2001) reported easy expulsion of placenta after delivery by using approximately 2 kg of pearl millet (*Pennisetum americanum*) grain, 100g of methi (Trigonella foenum-*graecum*) seeds, 50 g of asalio (*Lepidium sativum*), 25 g of suva (*Anethum graveolens),* and 500 g of jiggery boiled in water for one hour. The cooked feed (after cooling) was fed to cows after delivery. Significantly lower days to first AI were recorded in supplemented groups (Table 1). Similar findings were reported by Mendoza et al. (2011), who found that feeding sunflower oil during pre-partum reduced the days to first insemination. Significantly ($p<0.05$) lower days open T1 (81.0± 1.0), T2 (88.875±6.22), and T3 (78±9.9) than the control group (143±±14.29).

Lesser days open were an important marker in terms of reproductive efficiency. Incorporation of lipids in rations for dairy cattle usually increases the energy density of the ration and improves lactation and reproductive performance (Funston, 2004). However, when they are supplied in early lactation in greater proportion, frequently there is a reduction in feed intake due to a reduction in dry matter digestibility and an increase in energy availability, so when lipids are supplied in the early postpartum period, there is little alteration in the energy status of the animal even when a higher energy density ration is consumed (Santosh et al*.,* 2009). There was a significant improvement in conception rate in supplemented groups T1 (57%), T2 (50%), and T3, (62.5%) compared to the control group (38%). These findings reveal a better conception rate in the supplemented group than the control, probably due to improved energy balance. The present study is corroborated by Rajkumar et al*.* (2004), who found that the effect of *Saraca asoca* stem bark and *Trigonella foenum-graceum* seeds on reproductive performance and studied serum progesterone and micromineral profiles in anestrus cows and reported that the percentage of animals induced in estrus and overall pregnancy rate using fenugreek seeds were higher (83.33 and 80.00 %) as compared to *Saraca asoca* (66.66 and 50.00%) at doses of 50 and 100 g. The results of the present study, corroborated by Armstrong et al. (1990), Burke et al. (1996), Carroll et al. (1994), and Ferguson et al. (1990), reported an improvement either in the first AI service conception rate or in the overall rate of conception or pregnancy with the supplementation of fat. The incidence of metritis was lower in the supplemented groups (T1-20%, T2-10%, and T3-10%) as compared to that of the control group (T0-30%). Berhane (2000) reported that the highest reproductive performance (onset of estrus, pregnancy rate) was recorded in dairy cows supplemented with gur, line seed oil, and maithy (fenugreek) mixtures. The present result was in line with the reports of Dematawewa and Berger (1997) and Santosh (2009).

The effect of supplementation of mustard oil, poly-herbal mixture, and mustard oil plus poly-herbal mixture on milk yield parameters is shown in Table 2. Milk yield was significantly ($P<0.05$) higher in the T2 and T3 groups in comparison to the T0 and T1 groups. A similar finding was observed by Dai et al. (2004) on Holstein dairy cows.

Milk fate%, milk protein%, milk SNF%, and total solids% showed no significant difference between the supplemented (T1, T2, and T3) and control (T0) groups (Table 2). These findings were similar to those reported by Mirzaei et al. (2012) and Chandra et al. (2017) in the case of cross-bred goats supplemented with herbal mixtures in the supplemented groups and the control group.

The effect of the mustard oil, poly-herbal mixture, and mustard oil plus poly-herbal mixture on NEFA concentrations from 21 days pre-calving to 21 days post-calving is shown in Table 3. The mean values of NEFA concentrations were significantly ($P<0.05$) lower in T1 and T3 in comparison to the T0 group. From the 21st day pre-calving to the day of calving, the NEFA concentration rises; thereafter, it begins to

Table 1. RFM, time required for expulsion of fetal membranes, days to first insemination, days open, conception rate and metritis in Sahiwal cow fed on diets supplemented with mustard oil, poly-herbal mixture and mustard oil + poly-herbal mixture during the transition period

Particular	Mustard oil (T1) (n=10)	poly-herbal mixture (T2) (n=10)	Mustard oil + poly-herbal mixture (T3) (n=10)	Control (T0) (n=10)
Incidence of RFM (%)	20	10	10	30
Time required for expulsion of fetal membranes	4.948a±0.21	5.847b±0.16	4.065b±0.16	9.406c±0.22
Days to first insemination (days)	67.66a±6.37	73.44a±6.79	62.22a±3.53	114.11b±14.04
Days open (days)	81.25a±10	88.875a±6.22	78a±9.9	143b±14.29
Conception rate (%)	57	50	62.50	38
Incidence of Metritis (%)	20	10	10	30

Means bearing different superscripts in same row differs significantly (P< 0.05)

Table 2. Overall mean of milk yield and milk composition in Sahiwal cows fed on diets supplemented with mustard oil, poly-herbal mixture and mustard oil + poly-herbal mixture during the transition period

Particular	Mustard oil (T1) (n=10)	poly-herbal mixture (T2) (n=10)	Mustard oil + poly-herbal mixture (T3) (n=10)	Control (T0) (n=10)
Milk (kg/d)	8.756a±0.26	9.374b±0.34	9.796b±0.15	8.380a±0.22
FCM (6%)				
Fate (%)	4.97±0.077	4.63±0.083	4.88±0.083	4.58±0.113
Protein (%)	3.25±0.037	3.22±0.050	3.43±0.043	3.09±0.070
Lactose (%)	4.80±0.045	4.71±0.063	4.82±0.047	4.55±0.030
SNF (%)	9.08±0.130	9.23±0.147	9.19±0.160	8.91±0.097
Total solids (%)	13.99±0.207	13.76±0.230	14.00±0.243	13.50±0.210

Means bearing different superscripts in same row differs significantly (P< 0.05)

Table 3. Plasma NEFA concentration (µmole/L) in Sahiwal cows fed on diets supplemented with mustard oil, poly-herbal mixture and mustard oil + poly-herbal mixture during the transition period

Period (days)	Mustard oil (T1) (n=10)	poly-herbal mixture (T2) (n=10)	Mustard oil + poly-herbal mixture (T3) (n=10)	Control (T0) (n=10)
-21	125.609aD±1.35	136.201aD±0.47	121.727aD±0.43	136.048aD±0.81
-15	217.918aC±9.37	310.88bC±0.72	205.453aC±0.63	311.445bC±6.08
0	564.295bA±5.89	632.502cA±1.06	524.7136aA±1.59	631.49cA±3.53
15	334.054aC±8.47	393.682bD±1.40	306.553aC±0.66	399.316bD±14.2
21	220.318aB±8.68	265.173bB±0.40	204.971aB±0.36	265.878bB±6.15
Over all Mean±SEM	292.439a±6.752	347.688b±0.81	272.684a±0.734	348.835b±6.154

Means bearing different superscripts within a row (small later) and column (capital later) differ significantly (p≤0.05)

Table 4. Plasma BHBA concentration (µmole/L) in Sahiwal cows fed on diets supplemented with mustard oil, poly-herbal mixture and mustard oil + poly-herbal mixture during the transition period

Period (days)	Mustard oil (T1) (n=10)	poly-herbal mixture (T2) (n=10)	Mustard oil + poly-herbal mixture (T3) (n=10)	Control (T0) (n=10)
-21	342.855bD±3.7	368.896cD±0.63	322.757aD±2.01	371.561cD±2.46
-15	407.927 bC±3.08	478.217cC ±0.92	384.56aC±1.32	484.831cC ±2.52
0	649.925bA±2.4	763.582cA±0.83	610.445aA±2.19	765.889cA±.5.64
15	422.372bD±2.02	480.666cC±1.26	407.095aD±1.48	482.573cC±1.48
21	526.794bB±2.55	576.432cB±0.57	479.559aB±1.98	578.548cB±1.98
Over all Mean±SEM	469.975b±2.75	533.559c±0.842	440.883a±1.79	536.680c±2.816

Means bearing different superscripts within a row (small later) and column (capital later) differ significantly (p≤0.05)

decline in all groups. Table 3 shows that on days -15, 0, 15, and 21, the T1 and T3 groups had much lower NEFA concentrations (p≤0.05) than the control (T0) group. Increased concentration of NEFA indicates body fat mobilization in response to increased energy demand. Various studies indicate that NEFA levels in blood plasma are as biomarker of energy balance; when the supply of glucose is insufficient to meet energy demands, its level increases, especially pre-calving. Oetzel (2004) reported that NEFA concentration less than than 400 µmole/L indicates problems with energy balance and subsequent intensive lipomobilization; similar findings were reported by Chiesa et al. (1991), Prasad and Singh (2010) and Jackson et al. (2011). It is increased during early lactation. This suggests that the treatments applied to the T1 and T3 groups were effective in regulating NEFA levels during this critical period. Furthermore, the significant differences observed indicate the potential benefits of these treatments in improving metabolic health around calving. Similar results were reported by Singh et al. (2014) in the feeding of prill fat and Mendoza et al. (2011) in the feeding of fish oil.

Table 4 shows the effect of mustard oil, polyherbal mixture, and mustard oil + polyherbal mixture on BHBA concentrations between 21 days before and 21 days after calving. The mean BHBA concentrations were significantly (P<0.05) higher in the T0 groupthan in the T1 and T3 groups. From the 21st day pre-calving to the day of calving, BHBA concentrations increased; thereafter, they began to decline in all groups. There was a significantly (p≤0.05) higher BHBA concentration in the T0 group than in the T1 and T3 groups on days -15, 0, 15, and 21 of calving (Table 4). In the control group, the number of RFM cases was higher, and in RFM cases, the BHBA concentration was also comparatively higher. The elevated BHBA concentration on the day of calving in the control (T0) group reflected the stress conditions. Ketone body BHBA is a reliable indicator of lipid mobilization in ruminants. These findings indicate that the metabolic status of the T0 group may have been compromised, leading to higher BHBA levels during the critical periods around calving. Consequently, monitoring BHBA levels can provide valuable insights into the metabolic health of dairy cows during calving.

4. CONCLUSION

The age-old practice of supplementing mustard oil and poly-herbal mixture in animals is well known; however, novel findings of the present study depict that supplementation of mustard oil (200 g), poly-herbal mixture (@425 g), as well as mustard oil and (200 g) poly-herbal mixture (@425 g) in combination may be used safely during the transition period to improve energy balance, which subsequently helps in improving the post-partum reproductive efficiency by reducing days open, days to first insemination, increasing the conception rate, and improving the productive performances of Sahiwal cows.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author (s) hereby declare that NO generative AI technologies such as Large Language Models and text to image generators have been used during writing and editing of this manuscript.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

ACKNOWLEDGEMENT

The authors are highly thankful to the Director of ICAR-National Dairy Research Institute (NDRI) for providing financial support and necessary facilities for carrying out the research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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