Original Research Article

**Impact of adding poly-herbal preparations and mustard oil supplements on the reproductive and production performance of Sahiwal cows**

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ABSTRACT

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| **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% andT3-10%) 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 control group. 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 230.58 million tons per year (2022-23). 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. Nutritional supplements during this period may therefore provide some support to overcome the adverse effects of impaired metabolic and immune functions. 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 (Dhimanet al., 2000), 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 (Nemadeet al., 1994).**

2. material 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 Trigonellafoenum-graecum (Methi) 25g, Trachyspermumammi (Ajwain) 25g, Foeniculumvulgare (Saunf) 25g, Anethumgraveolens (Sowa) 25g, Zingiberofficinale (Sundh) 25g, Elettariacardamomum (Bari elaichi) 25g, Kala namak 25g, and 250g Jaggery . The polyherbal mixture was prepared by mixing each herb ingredient, making a total quantity of 150 g, and 25g 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 (Pennisetumamericanum) grain, 100g of methi (Trigonellafoenum-graecum) seeds, 50 g of asalio (Lepidiumsativum), 25 g of suva (Anethumgraveolens), 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 (Santoshet 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 Rajkumaret al. (2004), who found that the effect of Saracaasoca stem bark and Trigonellafoenum-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 Saracaasoca(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%, andT3-10%) 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, lineseed 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 Mirzaeiet al. (2012) 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 BHBA and NEFA concentrations is shown in Table 2. The mean values of BHBA and NEFA concentrations were significantly (P<0.05) lower in T1 and T3 in comparison to T0 and T2 groups. There was no significant difference observed between the T0 and T2 groups. An increased concentration of BHBA and NEFA indicates body fat mobilization in response to increased energy demand. Various studies indicate that BHBA and NEFA levels in blood plasma act as biomarkers of energy balance. When there is not enough glucose available to meet energy needs, especially before calving, demands on glucose levels increase. Oetzel (2004) reported that NEFA concentrations less than 400 µmole/L indicate problems with energy balance and subsequent intensive lipomobilization. The results of the present study corroborated the findings of Chiesaet al. (1991), who stated that there was an increase in NEFA during early lactation.  Singh et al. (2014) reported that NEFA levels decreased in prill fat supplementation group cows as compared to the non-supplemented group.**

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 (200g), poly-herbal mixture (@425 g), as well as mustard oil and (200g) poly-herbal mixture (@425g) 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.**

Ethical approval (whereever applicable)

 “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.”

**COMPETING INTERESTS DISCLAIMER:**

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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

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| **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 ofmilk yield, milk composition, BHBA and NEFA in Sahiwal cowsfed on diets supplemented withmustard oil, poly-herbal mixture and mustard oil + poly-herbal mixture during the transition period.

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| --- | --- | --- | --- | --- |
| **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 |
| 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 |
| BHBA, mmol/l | 469.975b±2.75 | 533.559c±0.842 | 440.883a±1.79 | 536.680c±2.816 |
| NEFA, mmol/l | 292.439a±6.752 | 347.688b±0.81 | 272.684a±0.734 | 348.835b±6.154 |

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