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Shraddha Barjibhe
Veterinary Assistant Surgeon, Bagicha, Dist- Jashpur, Chhattisgarh, India

PS Oberoi
Division of Livestock Production & Management, ICAR- National Dairy Research Institute, Karnal, Haryana, India

Brijesh Patel
Assistant Professor, Livestock Production & Management, College of Agriculture and Research Station, Raigarh, Chhattisgarh, India

Pankaj Kumar Patel
Division of Medicine, ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India

Correspondence
Brijesh Patel
Assistant Professor, Livestock Production & Management, College of Agriculture and Research Station, Raigarh, Chhattisgarh, India

Improving reproductive efficiency through the supplementation of mustard oil, poly-herbal mixture and butyric acid during the periparturient period in Sahiwal cows

Shraddha Barjibhe, PS Oberoi, Brijesh Patel and Pankaj Kumar Patel

Abstract
Periparturient period is a challenging time for dairy cattle as the cows have to deal with physiological, metabolic and endocrine changes along with environment and management related stresses. Present study was conducted to know the effect of mustard oil, butyrate and polyherbal mixture supplementation on productive and reproductive performance of Sahiwal cows. For this 20 multiparous Sahiwal cows were selected and divided into two equal groups according to their EPA. The cows were fed with scheduled NDRI ration but in addition, cows of treatment group were fed mustard oil (200 ml/day) and polyherbal mixture (150g/day) 30 days before and after parturition whereas butyrate (200g/day) was fed 30 days post-partum and decoction for 7days postpartum. Blood sampling was done on 28th, 21st, 14th, 7th and 3rd day prepartum, on the day of calving and 3rd, 7th, 14th, 21st and 28th day postpartum. Ultrasonography of uterus to see the uterine involution was done up to 42 days postpartum in weekly interval. The mean NEFA values were significantly (P<0.05) lower in supplemented group as compare to control group. The average value of uterine diameter and cervical diameter during postpartum period was significantly (P<0.05) lower in supplemented group than control group. There were 20% cases of RFM in control group whereas in supplemented group there were no cases of RFM. The time taken for expulsion of fetal was significantly (P<0.05) lower in supplemented group. Days to 1st observed heat were significantly less in supplemented (P<0.05) group. Birth weight of calf was significantly higher in supplemented group. Conception rate was higher in supplemented group. The mean days to 1st service was significantly (P<0.05) lower in treatment cows as compare to control group. The results of the study help to conclude that the supplementation was helpful for maintaining overall reproductive efficiency by improving reproductive health.

Keywords: Conception rate, estrus, involution and Sahiwal

1. Introduction
In order to get high reproductive efficiency in the dairy herd, it is required that each cow should calve during a pre-planned calving season, with an optimum calving interval that maximises the economic output. It is a point of concern that reproductive efficiency in the dairy cow has been decreasing in the past two decades. The cause of low fertility is multifactorial, involving genetic improvement, inadequate nutrition, poor reproductive management, an increased incidence of diseases and overall poor cow welfare [1]. Nutritional status of dairy cattle may impact reproductive performance, with the best-documented relationship between energy status and reproductive performance [2]. Negative energy balance can affect cow reproductive performances through some biological mechanisms like; metabolic, hormonal modifications regulated by pituitary-hypothalamic axis (as LH, FSH, GH, insulin, leptin, IGF-1, oestrogen and progesterone), interactions between blood metabolites and ovarian activity (glucose, NEFA, BOHB) and relationship between uterine functionality and immune response during pregnancy and transition period [3]. The cow is most likely to undergo in negative energy balance during the transition period. As the period of transition between late pregnancy (-3 weeks) and early lactation (+3weeks) presents a huge metabolic challenge in terms of energy metabolites and hormonal changes [4]. As parturition approaches, there is a progressive decrease in dry matter intake, with a decrease of about 30% in the last 3 weeks of gestation and almost 90% of that decrease occurring during the 5 to 7 days before calving [5]. After calving, most of the energy gets diverted for colostrum and milk synthesis as a result of which animal goes into the negative energy balance [6].

Therefore as postpartum health has a dramatic impact on fertility of dairy cows, it is pivotal to take care of nutrition to improve the reproductive performance of dairy cows.

Fat is an energy dense nutrient. Improved reproductive performance and reduced incidence of reproductive disorders have been observed by feeding supplemental fat to dairy cows [7]. Mustard oil is one of the cheapest fat supplement (8-10%) been used traditionally in feeding of Indian dairy cows and buffaloes, which contain 60% MUFA and 40% PUFA. Short Chain Fatty Acids (SCFA) like butyric acid are involved in improving immune processes, mostly as anti-inflammatory agents [8]. It is also reported to be an incredible source of energy, increase insulin sensitivity and functions of mitochondria [9]. Supplementation of anti-oxidant and immune-modulating agent during transition period has been reported to be resulting in early onset of postpartum estrus and improved pregnancy rate [3]. Many herbs have antioxidant and immune modulatory activity. These herbs have been used since Pre-Vedic times because they are safe to use, cheap and easily available, have no side effect and no residual effect on milk [10]. *Asparagus racemosus, Ocimum sanctum, Trigonella foenum-graecum Trachispermum ammi,* Foeniculum *vulgare* have been scientifically validated as a reproductive system tonic, immunomodulator, antioxidant, and anti-stress agent [11, 12]. Therefore, by keeping these points into mind current study was designed to see the effect of supplementing mustard oil, polyherbal mixture and butyrate on reproductive efficiency in Sahiwal cows.

2. Materials and Methods

2.1 Study area

The present study was conducted at Livestock Research Centre of National Dairy Research Institute (N.D.R.I.), Karnal - Haryana, India. The NDRI, Karnal is located on 29^0 43' N latitude and 76^0 58' E longitudes at an altitude of 245 meters above the mean sea level in the bed of Indo-Gangetic alluvial plain. The maximum ambient temperature in summer goes up to 45 ^{0}C and minimum temperature in winter comes down to 00C with a diurnal variation in the order of 15-20 ^{0}C.

2.2 Animals

For the present study, 20 multiparous Sahiwal cows of 2-6 parity were selected from Livestock Research Center of NDRI, Karnal. All the selected animals for study were free from any anatomical, physiological and infectious disorders and require less than 5 services per conception. On the basis of lactation no. and expected producing ability (EPA), cows were divided into 2 groups of 10 cows in each group so that each group had nearly similar average producing ability.

2.3 Management of experimental animals

The experimental animals were kept in separate pens during the experiment. Control cows fed standard ration of institute for pregnant and lactating cows. Cows under treatment were fed Mustard oil (200ml/cow/day) and Poly Herbal Mixture (150/cow/day) for 60 days (30 days before and after parturition), decoction immediately after parturition for 7 days and Sodium butyrate (200g/cow/day) for 30 days postpartum. All cows were fed on isoenergetic diet formulated to meet or exceed the predicted requirements of National Research Council (NRC, 2001) and offered fresh chaffed green fodder ad libitum provided in three to four meals throughout the day. Decoction is prepared by mixing all the ingredient were mixed with 1l water and then boiled for 5-10 minutes.

2.4 Blood collection and analysis

Blood samples were collected from jugular vein in 10 ml heparinized (20 IU heparin/ml blood) tube at 7:30am before offering any feed on -28, -21, -14, -7, -3, 0 as well as on +3, +7, +15, +21 and +28 day after calving for blood metabolite estimation. For hormone analysis blood samples were collected on 7^{th}, 14^{th}, 21^{th}, 28^{th}, 35^{th}, and 42^{nd}day after calving. The plasma was harvested within one hour after sampling following centrifugation at 2500rpm for 30 minutes at 4°c and plasma collect and store at -20°c in the storage vials of 2 ml capacity till the analysis of blood metabolite and hormone.

The copper soap solvent extraction method modified by Shipe *et al.,* (1980) was adopted for the estimation of plasma NEFA. The plasma progesterone concentration were estimated using Enzyme Linked Immunosorbent Assay (ELISA) Kit for progesterone, (Lot: L151125848) from Cloud-Clone Corp.

2.5 Reproductive parameters

The incidence of various reproductive disorders in treatment and control animals was recorded. The cows that had not expelled the fetal membrane within 12 h after parturition [13] were considered as cases of retention of fetal membranes (RFM). After parturition all experimental animals were observed regularly for first symptom of heat during both morning and evening. The interval from parturition to first symptom of heat was recorded to determine days from calving to first heat. The interval between calving to successful conception was recorded as service period. Conception rate was defined as the proportion of animals gets conceived out of total inseminated animal. All these parameters were determined by records taken from Livestock Research Centre, NDRI, Karnal.

2.6 Ultrasonic examination of uterus during postpartum period

For confirming cervical and uterine involution, the organs were examined by transrectal ultra-sonography at 7^{th}, 14^{th}, 21^{st}, 28^{th}, 35^{th} and 42^{nd} day postpartum. The transversal diameters of the involuting uterine horn and cervix were measured using a real-time B-mode ultrasound scanner. Uterine involution was considered to be complete when no further reduction in the uterine diameter for three successive examinations was recorded, in addition to absence of lochia in the uterus [14].

2.7 Statistical Analysis

Differences in the parameters among dietary treatments were analyzed by Analysis of variance (ANOVA), homogeneity test and Duncan's Multiple Range Test, (DMRT) using IBM-SPSS and Sigma Stat 3.1 software packages.

3. Results and Discussion

3.1 Birth Weight of Calf

The mean birth weight of calf was significantly (P<0.01) higher in treatment cows as compare to control group (Fig 1). Birth weight of calf has positive correlation with dam's energy status during prepartum period. So the normal energy state of oil supplemented group might have the reason for better birth weight. Fat supplementation lowers the insulin level and the lower insulin level have glucose sparing effect. So this spared energy (in terms of spared glucose) might have diverted towards the fetal growth. Therefore the birth weight of calf was higher in supplemented group. The result corroborated the findings of Bajhau and Kennedy, (1990) [15],

Skjevdal, (1979) [16].

As greater negative energy balance is associated with reduced reproductive performance [17]. In our study energy sources like oil, butyrate and jaggery were provided. So the better conception rate in the treatment group of animals was may be due to the better energy status (as indicated by NEFA cons.), as they were supplemented with energy sources.

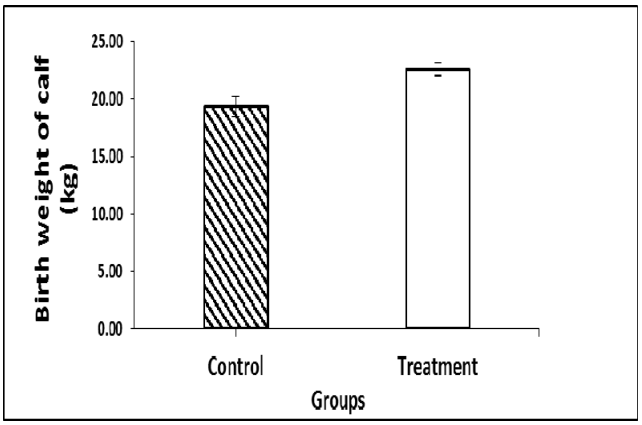

Fig 1: Average birth weight (kg) of calf in different groups during postpartum period

3.2 Plasma NEFA Concentration

The plasma NEFA concentration varied significantly between the group ($P<0.01$) and between periods of the experiment ($P<0.01$) (Fig 2). The average value during prepartum was 309.36±5.52 and 291.76±6.31 µM/L in control and supplemented group, respectively. On the day of parturition the NEFA levels were high 436.67±3.36 and 349.0± 9.98 µM/L in control and supplemented group respectively and differ significantly ($P<0.05$). The postpartum overall NEFA levels were 347.3±7.98 and 312.33±7.73 µM/L in control and supplemented groups, respectively ($P<0.01$). The results of present study corroborated to Chiesa *et al*. (1991) [18] who stated that there is increased in NEFA during parturition and few days after parturition.

NEBAL improvement due to increase energy density through fat supplementation, which also might adapt liver to bear large prepartal increases of plasma NEFA and improve utilization of these substrates by peripheral tissues [19]. Feeding fat supplemented diets near the time of calving has been suggested to decrease liver triglycerides after parturition [20]. This strategy assumes that dietary fatty acids are incorporated into intestinally synthesized lipoproteins, and in contrast to NEFA, are metabolized predominantly by extra-hepatic tissues. It is also assumes that the energy status of the animals will be improved by fat supplementation. Consequently fat mobilization from adipose tissues will be reduced.

Fat supplementation decreases the leptin concentration prepartum. Leptin is a hormone which regulate the hunger. When leptin concentration is higher it stimulate satiety centre. So due to fat supplementation as the leptin concentration decreases it stimulate to take more feed and thus maintains the energy balance.

Various studies indicate that NEFA levels in blood plasma as biomarker of energy balance, when the supply of glucose is insufficient to meet energy demands its level increases specially before calving. Oetzel (2004) [21] reported that NEFA concentration more than 400µmol/L indicates problems with energy balance and subsequent intensive lipomobilization. So as the NEFA level around parturition were comparatively low in treatment group of animal it indicate that the supplementation maintained the energy balance in the same.

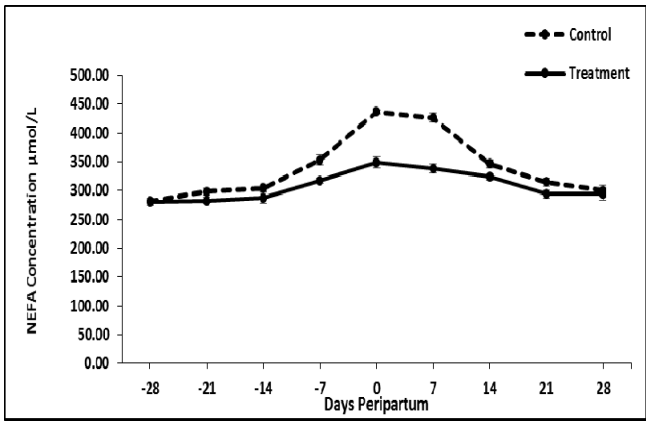

Fig 2: Plasma NEFA concentration µmol/l during peri-partum period in different group of cows

3.3 Retention of fetal membrane (RFM) and time required for expulsion of placenta

Out of Ten cows included in the control group, two cows (20%) had RFM, however, none of the cows in treatment groups had RFM. The time taken for expulsion of fetal membrane was significantly ($P<0.05$) less in treatment group of animals (Fig 3). Our result was in agreement with the observation of Ulfina *et al*. (2015) [22] who also reported that supplementation of butyrate help in expulsion of fetal membranes.

Immunosuppression is may be a cause of RFM [23]. To maintain pregnancy, it is necessary to suppress the immune response to save the fetus from rejection and RFM may be due to the result of failure of switching off this protective activity of immune system. So the anti-inflammatory and immunomodulatory effect of poly-herbal mixture and effect of SFCA (Butyric acid) in regulating neutrophil function and in modulating cytokine expression, especially in presence of inflammatory stimuli [8] might have helped in expulsion of placenta.

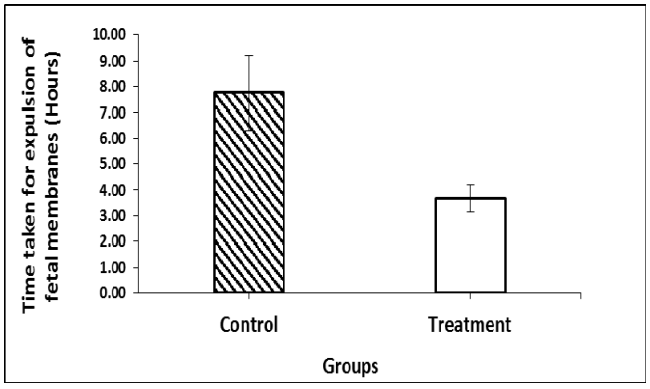

Fig 3: Time taken for expulsion of fetal membranes (Hours)

3.4 Uterine involution

There was an early ($P<0.05$) cervical and uterine involution in treatment group of cows than the cows of control group (Fig 4 and 5).

Poor reproductive health and uterine infections delays the uterine involution. In negative energy balance condition, the increased plasma levels of non-estrified fatty acids (NEFA)

impair the migration, phagocytic and killing activity and/or the oxidative burst of PMN and other leucocytes, enhancing the susceptibility of host to invading pathogens [24] that means reducing immunity. So as the NEFA level is significantly (P<0.01) lower in treatment group of cows which is suggestive of better energy status of treatment cows. Therefore the low level of immunosuppression is might be the reason of early uterine involution.

Retained fetal membrane delays the uterine involution and predispose the animal to endometritis or metritis and decrease fertility [25]. So as in some animals of control group there was RFM and the time taken for expulsion of fetal membrane was also higher in the same, so the uterine involution was late.

Administration of *Asparagus racemosus* based herbal formulation increased wet and dry uterine weight with marked increased in estrogen but not progesterone level as compared to control rats [26]. Elevated estrogen level stimulates and repair uterine endometrium tissue and depot uterine glycogen. It has been reported that decrease in the estradiol 17-beta/ 17-alpha ratio resulted in a reduced rate of release of prostaglandins from the uterus and a slower rate of uterine involution [27]. Besides, a higher concentration of biologically active estrogens may result in a faster rate of uterine involution due to increased PGF2 alpha release and *vice-versa* [28]. The lower incidence of postpartum reproductive disorders observed in the present study might be a reason for early uterine involution in supplemented animals. It was hypothesized that endometrial damage due to infection immediate after parturition reduced release of PGF2 alpha and consequently delayed involution [29].

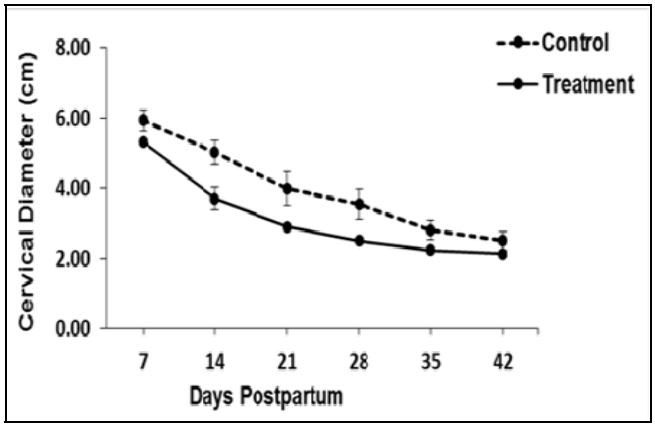

Fig 4: Average Cervical Diameter (Cm) In Different group of cows during postpartum Period

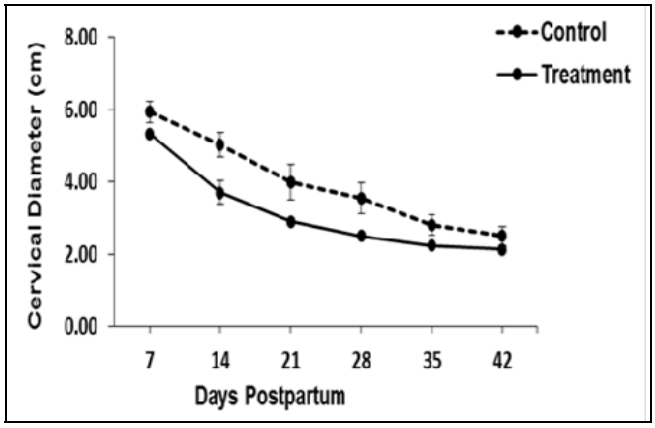

Fig 5: Average uterine diameter (cm) in different group of cows during postpartum period

3.5 Days to First Observed Heat and first heat

The mean value of days to first observed heat was significantly (P<0.05) lower in treatment group as compare to control group (Fig 6).

Earlier first postpartum estrus in supplemented groups could be due to estrogenic property of *Asparagus racemosus* which might have stimulated the ovarian function and uterine tonicity properties of *Asparagus racemosus* that could have helped in early uterine involution and consequently early initiation of estrus cycle in supplemented groups [30]. The findings were in accordance with the earlier reports [31]. Morrow *et al*. (1966) [29] also stated that early uterine involution is directly related to early estrus cycle initiation.

The mean days to 1st service was significantly (P<0.05) lower in treatment cows as compare to control group. The result corroborated findings to Mendoza *et al*., (2011) [32] who have reported feeding sunflower oil during pre-partum reduces the days to first insemination.

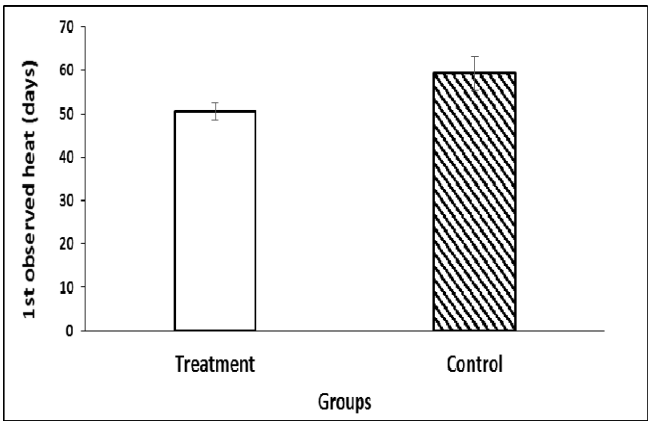

Fig 6: 1st observed heat (days) in different group of cows during postpartum period

3.6 Days Open (Service Period) and Conception Rate

The overall mean for days open was 65.5± 11.39 and 95± 18 treatment and control cows, respectively (Fig 7).

Shorter service period is associated with early uterine involution, better uterine health and earlier postpartum insemination. Butyrate helps in expulsion of fetal membranes which might have facilitated early uterine involution. The anti-inflammatory properties of butyrate might have helped to improve uterine health. Some of the herbs like Shatavari and Fennel have estrogenic properties which might have helped for earlier commencement of postpartum estrus. So the combined effect of supplementation results in shorter service period in treatment group of animals.

Conception rate in different groups have been depicted in (Fig 8) respectively. The conception rate of treatment group was greater than cows of control groups. The conception rate for treatment group was 50% whereas for control group it was 20%.

Results of Present study corroborated with 4 studies Armstrong *et al*. (1994) [30], Burke *et al*. (1996) [33], Carroll *et al*. (1990) [34] and Ferguson *et al*. (1990) [35] who reported an improvement in first service conception rate as well as in the overall conception rate on the supplementation of fat.

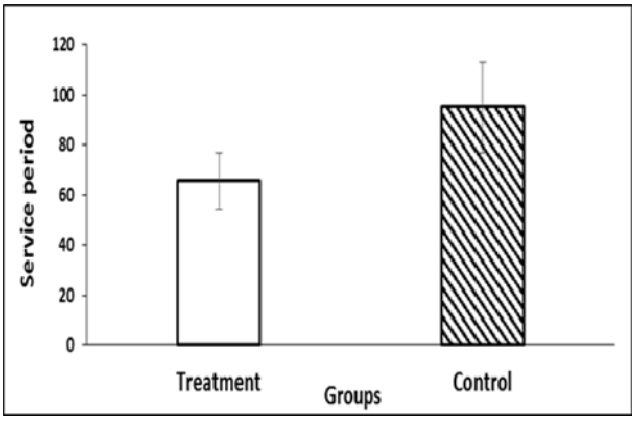

Fig 7: Days open in different group of cows during postpartum period

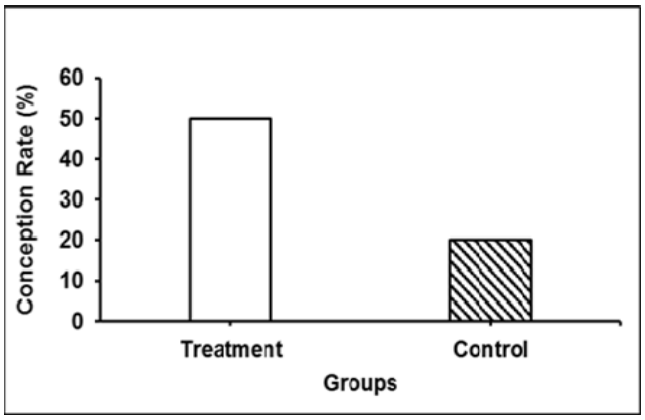

Fig 8: Conception Rate in different groups during postpartum period

4. Conclusion

Our study revealed that that supplementation reduced the time taken for expulsion of fetal membranes. There was an early cervical and uterine involution in treatment group of cows than the cows of control group. The days to 1st service was lower in treatment cows as compare to control group The conception rate of treatment group was greater than cows of control group. Overall reproductive efficiency was improved through supplementation as reproductive health was improved by supplementation

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