RESEARCH ARTICLE

Effect of Poly-Herbal Mixture and Butyric Acid on the Cleansing of the Uterus and Resumption of Ovarian Cyclicity in Murrah Buffaloes

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Abstract

The present experiment was conducted to evaluate the effect of a poly-herbal mixture and butyric acid supplementation, alone and in combination, on uterine cleansing of buffaloes during the transition period. Thirty-six Murrah buffaloes were selected 30 days before parturition and randomly divided into four equal groups with nine animals in each. The control group (T0) animals were fed diet as per farm feeding practice. Treatment groups T1, T2 and T3 were supplemented with poly-herbal mixture alone (T1), poly-herbal mixture + 200 ml butyric acid (T2) and with 200 ml butyric acid alone (T3) respectively. Postpartum involution of the uterus and cervix, fluid in the uterus, and ovarian follicular status were assessed by transrectal ultrasonography on the 7th, 14th, 21st, and 28th days. The blood samples were collected by jugular venipuncture into heparinized tubes at fortnightly interval from day 14 to day 42 postpartum; plasma was separated and stored under -20^{0}C until analyzed for progesterone estimation. The rate of uterine and cervical involution was significantly ($p< 0.05$) higher in supplemented groups compared to the control group. The proportion of buffaloes with large ovarian follicles within 28 days postpartum was also significantly ($p< 0.05$) higher in the treatment group compared to the control group. Plasma progesterone levels on day 35 postpartum in buffaloes under T_1 and T_2 group increased significantly ($p<0.05$) over the control group. The poly-herbal mixture facilitated early involution of the cervix and uterus, efficient cleansing of lochia, and improved subsequent fertility. It has potential to be used in dairy animals for improving postpartum reproductive efficiency.

Key words: Buffalo, Butyric acid, Cervix, Involution, Poly-herbal mixture, Transition period.

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Introduction

Reproductive efficiency is one of the major factors affecting milk production and the economic efficiency of the dairy farm. Delayed ovarian activity and reduced conception rates, lead to lowering the reproductive performance of dairy animals. Infertility in dairy animals causes tremendous economic loss to the farmers and the nation by reducing milk yield and the number of calves produced and by increasing the culling rate. It is estimated that around 18-40% of cattle and buffaloes were culled mainly due to infertility (Kaikini, 2002), which incriminates direct losses to the farmer as well as to the genetic resource.

India has a rich biodiversity of medicinal plants, which have been claimed to be effective in modulating reproduction, but the systemic scientific evidence regarding their mechanism of action, dosage, or clinical efficacy is lacking (Dajin *et al*., 1995). Therefore, to improve the immunity (Chandra *et al*., 2017) and reproductive health of dairy animals, dairy farmers traditionally use extracts of poly-herbal mixture made from various herbs like methi, ajwain, sanuf, sowa, sundh, barielaichi, after mixing with jaggery and black salt, it's commonly called as "karrah". The composition of the poly-herbal mixture varies and is largely based on individual farmers' experiences and beliefs. Users generally believe that the feeding of "karrah" helps the animal in the expulsion of the placenta and acts as a cleansing agent and improves the animal's reproductive performance. No scientific understanding existed for the use of Karrah.

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On the other side the information available for use of short-chain fatty acid (SCFA), especially butyrate, in ruminants showed that SCFA is an immunomodulator, anti-inflammatory, and an incredible source of energy (Jianping, 2013). A study conducted at NDRI on the role of short-chain fatty acids in the expulsion of bovine fetal membranes (Ulfina *et al*., 2015) also implied the beneficial effect of short-chain fatty acids (SCFA), particularly that of butyrate in modulating the postpartum reproductive disorders in dairy cows. However, scanty scientific information is available on the effect of "poly-herbal mixture" as well as "butyrate" feeding on reproductive performance in Murrah buffaloes. Hence the present study was undertaken to evaluate the effect of supplementing poly-herbal mixture and butyric acid on the uterine cleansing and ovarian follicular status in buffaloes.

Materials and Methods

The present study was conducted at Livestock Research Centre, ICAR-NDRI, Karnal on multiparous Murrah buffaloes (n=36).

Ethical Statement: The experimental plan and sampling procedure were duly approved by the Institutional Animal Ethics Committee of the institute. All the chemicals used in the study were procured from Sigma Chemicals Co. (St. Louis, Missouri, USA) unless specified.

Preparation of Polyherbal Mixture

Polyherbal mixture consisted of six herbs including seeds of *Foeniculum vulgare* (Saunf), *Trachyspermum ammi* (Ajwain), *Trigonellafoenum-graecum* (Methi), *Anethum graveolens* (Sowa), and *Elettaria cardamomum* (Cardamom), and rhizome of *Zingiber officinale* (Sundh). The polyherbal mixture was prepared by mixing 25 g of each herb making the total quantity of 150 g, and 25 g of black salt was mixed in 1 liter of water. This mixture was boiled till the half of water remained (for about 25-30 min), then 250 g Jaggery was added and further heated for 5-6 min. The above-prepared mixture was fed daily per head to the animal by mixing with the concentrate mixture.

Supplementation of the Polyherbal Mixture and Butyric Acid:

Thirty-six Murrah buffaloes were divided into 4 equal groups, control (T_0) and three treatment groups (T_1, T_2, and T_3) consisting of 9 animals in each group. The control group animals were fed diet as per farm practices (NRC, 2001, plus a 10% higher ration than the recommendations), Buffaloes of treatment group T_1 (BW 661.89±42.13 kg) were supplemented with the polyherbal mixture prepared as above for seven days postpartum from date of calving, treatment group T_2 (BW 664.22±14.81 kg) were supplemented as T_1, along with 200 mL butyric acid (99%) from 30 days before calving to 30 days after calving, and treatment group T_3 (BW 672.00±17.97 kg) were supplemented only with 200 mL butyric acid (99%) from 30 days before calving to 30 days after calving.

Ultrasonography and Rectal Palpation

Involution status was assessed through cervical diameter and uterine horn diameter by ultrasonography (Aloka UST-5820-5, Japan) on days 7, 14, 21, and 28 postpartum using a 6.5 MHz transrectal probe. The follicular development on both ovaries of all the buffaloes (small <3 mm; medium 3–6 mm and large >6 mm) was recorded in the course of ultrasonography. The entire uterus was scanned for the status of uterine fluid during the ultrasound examination (Fig. 1).

Estimation of Progesterone Concentration

Blood samples (9 mL) were collected from all thirty-six buffaloes on days 14, 21, 28, 35, and 42 postpartum by using jugular venipuncture in heparinized polystyrene tubes (Vacuette®, Greiner Bio-one Gmbh, Austria), and plasma separated out was stored at –20°C until analysed for progesterone hormone using ELISA Kit (Endocrine Technologies, Newark, CA, USA) following manufacturer's instructions.

Statistical Analysis

All data were subjected to ANOVA for randomized complete block design using the general linear model (GLM) of SPSS (SPSS, 2011) and group comparison was done by Turkeys' test. For the experimental groups, descriptive statistics was used to analyzed the data on cervical and uterine diameter and ovarian follicular status

Results and Discussion

Cervical diameter and uterine horn diameter from day 7 to day 28 postpartum are shown in Table 1. On day 7, 14, and 21 postpartum, there was a significant ($p < 0.05$) difference in cervical diameter and horn diameter among the treatment groups T_1, T_2, T_3, and control group. On day 28 of postpartum, there was a significant ($p< 0.05$) difference in cervical diameter between the supplemented T1, T_2 and T_3groups, and control group, the values were similar in all the supplemented groups. Similarly, Kaur (1998) opined that the estrogenic components of *Trigonella foenum-graecum* and *Trachyspermum ammi* improved the uterine tonicity eliciting prompt involution of the cervix and uterus. Lee *et al*. (2013) and Ho *et al*. (2011) also reported that herbal therapy is beneficial for the involution of the cervix and uterus and ovarian activity in postpartum dairy cows. We observed that both poly-herbal mixture and butyric acid facilitated the uterine recovery process and improved the uterine environment in parturient buffaloes.

On day 7 postpartum, as expected, all the experimental buffaloes (treatment T_1, T_2, T_3 groups and control group) had fluid accumulation in their uterus. However, the proportion of buffaloes with detectable fluid on day 28 postpartum was only 0%, 0%, and 22.2%, in the T_1, T_2, and T_3 supplemented groups, respectively, as compared to 77.8% in the control group. At any point of ultrasound examination during

postpartum period a significantly ($p< 0.05$) higher number of buffaloes had detectable fluid accumulation in the uterus in the control group as compared to treatment (T_1, T_2, & T_3) groups. To determine the health status of the uterus, the state of fluid accumulation in the uterus was assessed by ultrasound examination.

The proportions of animals having different types of ovarian follicles on different days postpartum in supplemented groups, and control (T_0) group are given in Table 2 and Figure 1. On day 14 postpartum, only one buffalo in treatment group T_2 (polyherbal mixture + butyric acid supplemented) had large follicle, while small follicles were observed in a considerable number of buffaloes in T_0, T_1, and T_2 groups. On day 21 postpartum, a majority of the buffaloes had small follicles in decreasing order to T_1, T_2, T_3, and T_0, and a considerable number of buffaloes had medium and large follicles. However, on day 28 postpartum, 88.89%, 66.67%, and 44.44% of buffaloes had large follicles in T_2, T_1, and T_3 treatment groups, respectively, while only 22.22% of buffaloes in the control group had large follicle. 88.89% and 66.67% of the buffaloes in the supplemented T_1 and T_2 groups, respectively, developed large follicle on day 28 postpartum indicating an early resumption of ovarian cyclicity in buffalo fed with a poly-herbal combination. Sahatpure *et al.* (2016) and Hameid *et al.* (2019) reported that the supplementation of Fenugreek contains phytochemicals play an important role in the pathophysiology of ovarian hyperstimulation in terms of size of the follicle in treatment group as compared to control group.

Dynamics of peripheral progesterone concentration in buffalo in supplemented and control group are shown in Table 3. Initially plasma progesterone concentration remained basal up to 14th day postpartum in both control group and supplemented groups. On day 28 & 42 progesterone levels were apparently higher and on 35 postpartum significantly higher ($p<0.05$) in buffaloes of T_1 and T_2 group than the control and T_3 groups. Moreover, in all the groups, plasma progesterone showed gradual increase with advancing postpartum period till day 35, establishing cyclicity, and the drop seen thereafter may be associated with follicular

Table 1: Weekly change in cervical and uterine diameter of Murrah buffaloes under different treatment groups during postpartum period

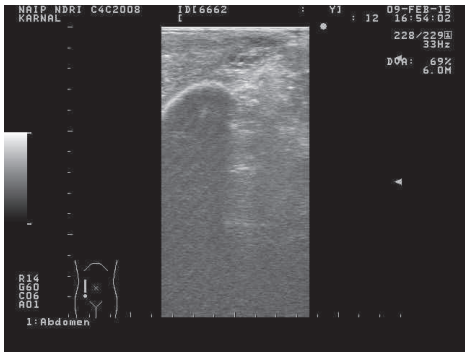
Weekly change	Days postpartum	Control (T_0)	Polyherbal mixture (T_1)	Polyherbal mixture + butyric acid (T_2)	Butyric acid (T_3)
Cervical diameter (cm)	7	14.29^{Ae}±0.15	10.37^{Be}±0.15	9.45^{Ce}±0.13	11.29^{De}±0.14
	14	10.29^{Af}±0.14	7.14^{Bf}±0.13	6.39^{Cf}±0.12	7.94^{Df}±0.13
	21	7.20^{Ag}±0.13	4.92^{Bg}±0.10	4.23^{Cg}±0.09	5.56^{Dg}±0.12
	28	5.15^{Ah}±0.11	4.24^{Bh}±0.08	4.23^{Bg}±0.09	4.25^{Bh}±0.11
Uterine diameter (cm)	7	12.29^{Aa}±0.15	8.67^{Ba} 0.15	7.95^{Ca}±0.13	9.49^{Da}±0.14
	14	8.69^{Ab}±0.14	5.84^{Bb}± 0.13	5.23^{Cb}±0.11	6.64^{Db}±0.13
	21	5.90^{Ac}±0.13	3.91^{Bc}± 0.10	3.43^{Cc}±0.09	4.56^{Dc}±0.11
	28	4.05^{Ad}±0.11	3.45^{Bd}±0.08	3.43^{Bc}±0.09	3.45^{Bd}±0.1

Means bearing superscripts in capital letter within the row and small letter within the column differ significantly ($p<0.05$).

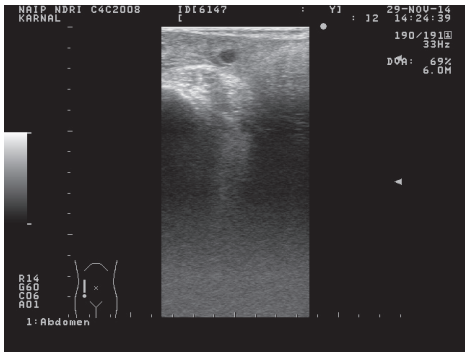
Table 2: Percentages of Murrah buffaloes having different types of ovarian follicles on different days postpartum in T_1, T_2 and T_3 and T_0 group (n = 9 each)

		% buffaloes with ovarian follicles on different days postpartum		
Parameter	**Groups**	**Day 14**	**Day 21**	**Day 28**
Small ovarian follicles	Control (T_0)	11.1	22.22	44.44
	Polyherbal mixture (T_1)	22.22	55.56	11.00
	Polyherbal mixture + butyric acid (T_2)	33.33	44.44	11.00
	butyricacid (T_3)	0.00	33.33	55.56
Medium ovarian follicles	Control (T_0)	0.00	11.11	22.22
	Polyherbal mixture (T_1)	11.11	33.33	22.22
	Polyherbal mixture + butyric acid (T_2)	22.22	55.55	00.00
	Butyric acid (T_3)	11.11	22.22	11.11
Large ovarian follicles	Control (T_0)	0.0	0.0	22.22^{a}
	Polyherbal mixture (T_1)	00.00	11.11	66.67^{c}
	Polyherbal mixture + butyric acid (T_2)	11.11	0.00	88.89^{d}
	Butyric acid (T_3)	0.00	11.11	44.44^{b}

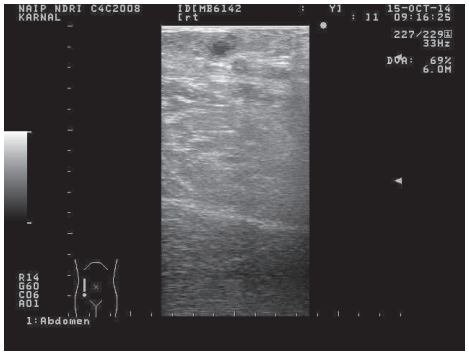
Means with different superscripts in a column differ significantly ($P <0.05$) between groups for a parameter.



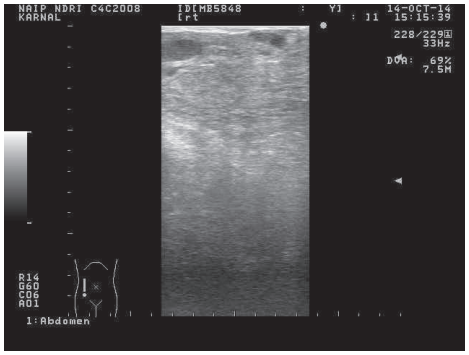
Control group (T_0)



Polyherbal mixture (T_1)



Polyherbal mixture + Butyric acid (T_2)



Butyric acid group (T_3)

Fig. 1: Ultrasonography of dominant follicle seen in control (T_0) and different treatment (T_1, T_2, T_3) groups of buffaloes.

Table 3: Plasma progesterone profile (ng/mL) in postpartum Murrah buffaloes in T_1, T_2 and T_3 supplemented groups and control (T_0) group (n=9 each)

Days postpartum	Control (T_0)	Polyherbal mixture (T_1)	Polyherbal mixture + butyric acid (T_2)	Butyric acid (T_3)
14	0.33±0.03	0.43±0.02	0.43±0.02	0.43±0.02
21	0.39±0.02	0.93±0.19	0.97±0.18	0.71±0.13
28	0.61±0.10	1.34±0.24	1.99±0.29	1.12±0.25
35	0.82^{a}±0.16	3.01^{b}±0.83	3.79^{b}±0.72	1.58^{ab}±0.44
42	1.01±0.25	2.82±0.85	2.61±0.92	1.74±0.47

Means bearing different superscripts within the row differ significantly ($p<0.05$).

phase of next cycle in some animals in each group. In line with our observations, Japheth *et al.* (2021) reported that the poly-herbal mixture is beneficial for early onset of cyclicity in postpartum dairy buffaloes.

The results depicted that the poly-herbal mixture and butyric acid improved the uterine environment in Murrah buffaloes. This could be because the many herbs in the mixture work together harmoniously. *Trigonella foenum-graecum, Foeniculum vulgare, Zingiber officinale*, and *Elettaria cardamomum* are some of the ingredients in the poly-herbal mixture supplement that have anti-oxidative and immunomodulatory properties (Sapra *et al.,* 2000; Ahmadiani *et al.,* 2001; Thangam and Dhananjayan, 2003; Bonjar, 2004; Jiang, 2006).

Conclusion

Studies have shown that supplementing with a polyherbal mixture and butyric acid during the transition period to buffalo promotes early involution of the cervix and uterus, effectively removes lochia, and promotes subsequent ovarian cyclicity and fertility.

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