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Influence of bypass fatty acid and *Tinospora cordifolia* supplementation on uterine involution, follicular development, and reproductive performance of Murrah buffaloes

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ABSTRACT

Murrah buffaloes were used to evaluate the influence of bypass fatty acid and *Tinospora cordifolia* supplementation on post-partum reproductive performance. Increased energy demand during early stage of lactation and decreased feed intake causes negative energy balance (NEB) that affects the reproduction and health of animals. In the present study, 20 healthy freshly calved Murrah buffaloes were taken from Livestock Research Centre of National Dairy Research Institute, Karnal and supplemented with bypass fatty acids and *Tinospora cordifolia* for a period of 90 days. Buffaloes were divided into 4 groups of five animals each, namely T1 (fatty acids), T2 (*Tinospora*), T3 (mix) and T0 (control) group on the basis of previous milk yield, parity and body weight. In T1 group, 150 g of bypass fatty acids; in T2 group, 150 g of *Tinospora cordifolia* stem powder and in T3 group, 150 g each of bypass fatty acids and *Tinospora cordifolia* stem powder was supplemented over and above the standard feeding schedule followed whereas no supplementation was provided to control (T0) group. Ultrasonographic (USG) monitoring of cervix and uterus revealed that the rate of involution of cervix and uterus was significantly higher in the treatment groups as compared to control group. Similarly, USG examination of ovaries showed that the size of follicles was significantly bigger in the supplemented groups. Buffaloes of supplemented groups had higher conception rate, and service period was significantly reduced in comparison to control group with early onset of post-partum first cyclicity. Based on these results, it can be concluded that combination of bypass fatty acids and *Tinospora cordifolia* supplementation improves the reproductive performance of lactating Murrah buffaloes.

Keywords: Buffaloes, Bypass fatty acid, Reproduction, Supplementation, *Tinospora cordifolia*

Maintaining reproductively healthy animal is one of the major requirements for profitable dairy farming. Post-partum reproductive management of animals is considered as a crucial component in farm animal management because it has a prominent role in determining profitability and efficiency of a farm (Leblanc 2008). Post-partum reproductive disorders lead to decreased productivity and economic losses to dairy farmers (Inchaisri *et al*. 2010). Soon after parturition, the energy requirement of dairy animals increases so as to meet the milk production potential, but the physiological stress and decreased feed intake limits the energy availability and ultimately leads to a negative energy balance (NEB). This NEB affects the production potential as well as makes the animal prone to health effects because of the compromised immunity (Singh *et al.* 2021).

Negative energy balance (NEB) and compromised immunity during post-partum are among the major factors for decreased reproductive efficiency of dairy animals (Nigussie 2018). Hence, reproductive performance of post-partum animals can be improved by incorporating high energy density supplement and immunostimulant in their ration. Supplements have been used since long to improve reproductive performance of dairy animals (Bindari *et al.* 2013). Fatty acids (Doreau *et al.* 2011, de Souza *et al.* 2021) and fats (Grummer and Carroll 1991, Garg *et al*. 2008, Khalil *et al.* 2012, Shelke *et al.* 2012) were found to exert a major impact on reproductive performance of dairy animals by improving the energy balance. Similarly, herbal supplements are also famous among farmers for improving reproductive efficiency of dairy animals as these herbs contain different photoactive compounds which are responsible for enhancing reproductive performance of dairy animals. Bypass fatty acid supplementation is an emerging concept as it involves supplementation of free fatty acids and leads to the availability of higher energy in comparison to bypass fat and other fat supplements. *Tinospora cordifolia* is an herbal supplement having immunomodulatory properties and contains different classes of photoactive componds like alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds, and polysaccharides (Kapil and Sharma 1997, Manjrekar *et al.* 2000, Mukherjee *et al.*

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2010, Aranha *et al.* 2012). This study was designed to study the combined effect of bypass fatty acids and *Tinospora cordifolia* supplementation on post-partum reproductive performance of Murrah buffaloes.

MATERIALS AND METHODS

Study area: Present study was performed at Livestock Research Centre (LRC) of National Dairy Research Institute, Karnal, Haryana, India from October 2019 to March 2020. The LRC is situated in the bed of Indo-Gangetic alluvial plain (29°42" N and 79°54" E longitudes) at an altitude of 245 m above the mean sea level.

Selection of animals and experimental design: For the present study, 20 freshly calved Murrah buffaloes were selected from Livestock Research Centre, NDRI, Karnal and further divided into four groups (T0, T1, T2 and T3) of 5 animals each on the basis of previous milk yield, parity, and body weight. It was ensured that the selected animals were free from any anatomical, physiological and infectious disorders. T0 was taken as control and buffaloes of this group were fed as per standard feeding schedule (ICAR 2013) whereas in T1, T2 and T3 along with standard feeding, supplementations were also given. About 150 g bypass fatty acid was supplemented (per buffalo per day) in T1, 150 g *Tinospora cordifolia* stem powder in T2 and a mixture of 150 g bypass fatty acids and 150 g *Tinospora cordifolia* stem powder in T3. Each buffalo was fed according to the respective treatment for a period of 3 months, i.e. from calving to 90 days of lactation. During the experimental period, buffaloes were kept under observation in loose type housing having availability of clean drinking water all the time. The experiment was conducted as per the guidelines of Institutional Animal Ethical Committee.

Housing and feeding management: The buffaloes were kept in loose housing system meeting all their space requirements as provided by Bureau of Indian Standard (BIS) and throughout the trial, they were tied for 1 h in a day for individual feeding of supplements as per treatment. Buffaloes were fed with seasonal green fodders grown in the institute like oats, maize, jowar, sugar graze and barseem and along with green fodder, maize silage or wheat straw was also offered. It was ensured that buffaloes got the required feed as per ICAR (2013) standards and clean drinking water was made available throughout day and night. Buffaloes under different groups were supplemented with bypass fatty acid and *Tinospora cordifolia* stem powder as per treatments.

Procurement of feed supplements: Bypass fatty acids were procured from the market (Carfe™ by Cargill) whereas *Tinospora cordifolia* was collected from Livestock Research Complex. The stems were collected and then cut into small pieces averaging about 2 cm and dried under shade. Authentication of the stem was performed by the ayurvedic doctor in the Institutional Health Complex. The stems were ground to powder form in a medicinal herb grinding machine, weighed, and packed in polythene bags.

Health management: All the buffaloes were monitored for any ailment before enrolling for the experiment. During the experimental period, routine health management of the farm was followed and buffaloes found sick were taken to Animal Health Complex of the Institute for the treatment.

Monitoring of involution status of uterus and cervix through transrectal ultrasonography (USG) and per rectal examination on day 35, 42, 49: Cervical and uterine involution was evaluated by subjectively measuring the thickness of the cervix and uterine horns with the help of USG. Measurements of cervix and uterine horns were taken at 35th, 42nd and 49th day of post-partum.

Monitoring of follicular status during post-partum period through transrectal ultrasonography (USG) and per rectal examination on day 35, 42, 49: The ovaries of all the buffaloes were scanned ultrasonographically on day 35, 42 and 49 post-partum to observe the *in vivo* changes in follicular development. Size and number of follicles on the ovaries were recorded.

Onset of first cyclicity: Onset of first cyclicity was marked by the presence of corpus luteum (CL) on the ovary. Day after parturition at which CL was spotted on the ovary by ultrasound examination was taken as day for onset of first cyclicity.

Days to first observed estrus: After parturition, all experimental animals were observed regularly for first symptom of heat during both morning and evening hours. The interval from parturition to first symptom of heat was recorded to determine days to onset of observed estrus.

Calving to first service interval: After calving when the animals were inseminated for the first time, that date was recorded and the interval between calving and the first service was calculated accordingly for all the experimental buffaloes.

Service period: Service period is the time period between calving and successful conception. Number of days from calving to successful conception was recorded for all experimental buffaloes.

Number of services per conception (NSC): Number of services per conception were recorded for individual group using the following formula:

$$\text{NSC} = \frac{(\text{No.of inseminations})}{(\text{No.of conceptions})}$$

Conception rate: Conception rate of the first service was calculated for individual group as per the following expression:

$$\text{CR} = \frac{(\text{No.of animal pregnant})}{(\text{No.ofanimal inseminated})} \times 100$$

Incidence of uterine infections based on cervical discharge: Buffaloes were screened for uterine infections at regular intervals during experimental period. Cervical discharge of the buffaloes was utilized for this purpose. It was taken in a blue sheath and any change in its colour, consistency was analyzed. Any deviation from normal colour or consistency was taken as a uterine infection.

Table 1. Effect of bypass fatty acid and *Tinospora* supplementation on involution status of cervix and uterus

	Control (T0)	Fatty acids (T1)	*Tinospora* (T2)	Mix (T3)
Day-35 (Post-partum)				
Cervix diameter (cm)	3.30^{a}±0.11	2.90^{ab}±0.12	2.93^{ab}±0.14	2.80^{b}±0.18
Right horn diameter (cm)	2.53 ^{a}±0.16	2.43 ^{ab}±0.08	2.38 ^{ab}±0.10	2.05 ^{b}±0.15
Left horn diameter (cm)	2.55 ^{a}±0.05	2.40 ^{ab}±0.15	2.35 ^{ab}±0.12	2.20 ^{b}±0.04
Day-42 (Post-partum)				
Cervix diameter (cm)	2.73±0.13	2.55±0.15	2.68±0.21	2.68±0.17
Right horn diameter (cm)	2.13±0.18	2.15±0.17	2.00±0.08	1.70±0.18
Left horn diameter (cm)	2.05±0.14	2.18±0.27	1.93±0.14	1.90±0.12
Day-49 (Post-partum)				
Cervix diameter (cm)	2.40±0.07	2.48±0.10	2.40±0.16	2.45±0.10
Right horn diameter (cm)	1.78±0.17	1.93±0.11	1.78±0.09	1.50±0.15
Left horn diameter (cm)	1.60±0.11	1.90±0.18	1.70±0.16	1.70±0.18

Values are Mean±SE of observations on five animals. ^{a,b}Values with different superscripts differ significantly in a row (P<0.05).

Table 2.Effect of bypass fatty acid and *Tinospora* supplementation on Follicular growth during post-partum

Follicles	Control (T0)	Fatty acid (T1)	*Tinospora* (T2)	Mix (T3)
Day-35(Post-partum)				
Small (2 to 4 mm), n	5.80±0.66	5.80±0.58	5.20±0.80	6.40±0.68
Medium (4 to 8 mm), n	0.80±0.37	0.60±0.24	0.40±0.24	1.00±0.45
Large (≥8 mm), n	1.00±0.45	0.80±0.37	1.20±0.20	0.80±0.37
Total number of follicles, n	7.60±1.08	7.20±0.49	6.80±0.97	8.20±0.58
Size of largest follicle, cm	1.13±0.02	1.13±0.02	1.18±0.04	1.20±0.00
Day-42(Post-partum)				
Small (2 to 4 mm), n	4.20±1.46	5.00±0.45	3.00±0.63	4.40±1.03
Medium (4 to 8 mm), n	0.60±0.24	1.00±0.45	0.60±0.40	0.80±0.37
Large (≥8 mm), n	0.40±0.24	0.80±0.20	0.80±0.20	0.80±0.20
Total number of follicles, n	5.20±1.46	6.80±0.49	4.40±0.24	6.00±1.18
Size of largest follicle, cm	1.16±0.05^{ab}	1.18±0.02^{ab}	1.13±0.04^{a}	1.28±0.06^{b}
Day-49(Post-partum)				
Small (2 to 4 mm), n	3.60±0.60	3.40±0.75	5.00±0.63	3.60±0.81
Medium (4 to 8 mm), n	0.20±0.20	0.40±0.24	0.40±0.24	0.40±0.24
Large (≥8 mm), n	0.80±0.20	0.80±0.20	0.60±0.24	0.80±0.20
Total number of follicles, n	4.60±0.81	4.60±0.93	6.00±0.71	4.80±0.73
Size of largest follicle, cm	1.17±0.04	1.25±0.05	1.16±0.02	1.25±0.05

Values are Mean±SE of observations on five animals in each. Value with different superscripts a, b differ (P<0.05) in a row.

Table 3. Effect of bypass fatty acid and *Tinospora* supplementation on post partum reproductive performance

Parameter	Control (T0)	Fatty acids (T1)	*Tinospora* (T2)	Mix (T3)
Onset of cyclicity (days)	49.60±2.20	47.20±2.31	46.20±1.71	44.80±1.71
First observed heat (days)	90.80±5.28	84.20±3.51	77.20±5.60	78.20±4.79
Calving to first service (days)	110.40±5.66	105.40±3.37	98.80±5.64	98.60±4.93
Service Period (days)	136.20^{b}±9.75	109.40^{a}±4.31	107.60^{a}±2.11	103.20^{a}±5.06
No. of services per conception	2.00±0.45	1.20±0.20	1.40±0.24	1.20±0.20
Incidence of reproductive disorder (n)	3	2	1	1
Conception rate of first artificial insemination (AI) (%)	40	80	60	80

Values are Mean±SE of observations on five animals in each. Value with different superscripts a, b differs significantly (p<0.05) in a row.

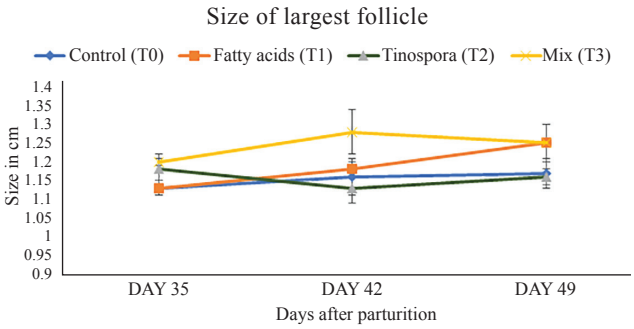

Fig. 1. Average size (cm) of follicles during day 35, day 42 and day 49 after parturition.

Statistical analysis: The data collected was analyzed using SPSS 22.0 (Statistical Package for the Social Sciences Version 22) software. Means and standard errors were calculated and one-way ANOVA test was applied to find out the statistically significant differences between groups. Further post-hoc Duncan test was applied to separate homogenous subsets.

RESULTS AND DISCUSSION

Effect of bypass fatty acid and Tinospora cordifolia *supplementation on cervical and uterine involution:* Measurements of cervical and horn diameters at day 35, 42 and 49 revealed that the rate of cervical and uterine involution was significantly affected by supplementation and it was faster in the treatment group in comparison to control group (Table 1). Cervical and uterine horn measurements showed a significant ($P<0.05$) difference between buffaloes of different groups at day 35, whereas the difference at day 42 and day 49 was non-significant ($P>0.05$) between different groups. Enhanced involution can be attributed to the improved energy balance and immunity of buffaloes through supplementation. Similar reports of enhanced rate of involution of uterus on supplementation of fat to dairy animals were reported by McNamara *et al*. (2003) and Khalil *et al.* (2012). Mallick and Prakash (2011) also reported faster uterine involution in crossbred cows fed with *Tinospora cordifolia*.

Follicular growth during post-partum: USG examination of ovaries onday 35, day 42 and day 49 showed the presence of different types of follicles (small, medium, large) on the ovaries of buffaloes and there was no significant ($P>0.05$) difference in total number of follicles in different groups. However, the size of largest follicles varies significantly ($P<0.05$) between groups on day 42. It was found that largest follicle was observed in T3 group on day 42. The values of different follicles and the size of largest follicles in different groups were enlisted in Table 2. The size of largest follicle at different days in different groups is also depicted in Fig. 1. Similar findings were presented by Bilby *et al.* (2006) and Garnsworthy *et al.* (2008) and they have stated that supplementation of polyunsaturated fatty acids (PUFA) increase the follicular size in dairy animals.

Effect of bypass fatty acid and Tinospora *supplementation on post-partum reproductive performance:* Different parameters were analyzed to assess the reproductive performance of buffaloes among different groups and it was found that supplementation of bypass fatty acid and *Tinospora cordifolia* significantly influence service period and conception rate. The average service period of the buffaloes was significantly reduced in treatment groups in comparison to the control group. It was 109.40±4.31 days in fatty acid (T1) group, 107.60±2.11 days in *Tinospora* (T2) group and 103.20±5.06 days in mix (T3) and 136.20±9.75 days in control (T0) group. The conception rate was significantly higher in the mix (T3) (80%) and fatty acid (T1) (80%) group followed by *Tinospora* (T2) group (60%) and it was least in control (T0) group (40%). Other reproductive parameters were also improved but significant difference was not there between different groups. The values of different reproductive parameters were presented in Table 3. Incidence of uterine infections were also reduced in *Tinospora* supplemented groups (T2 and T3) in comparison to other groups (T0 and T1) which may be due to the immunomodulatory properties of *Tinospora* leading to a healthy uterine environment and reducing the invasion of pathogens into reproductive tracts of buffaloes.The improved reproductive parameters can be attributed to improved energy balance and immunity status of buffaloes through supplementations and also bypass fatty acids and *Tionospora* have effect on hormones which play role in reproduction (Mallick and Prakash 201, Nirwan *et al.* 2019). The findings of this investigation were similar to that of Garg *et al*. (2008), Shelke *et al.* (2012) and Sharma *et al.* (2021) who also reported improved reproductive performance of dairy animals on supplementation of bypass fat and polyherbal mixture. Gowda (2014) also reported that the time required for appearance of first heat was reduced when animals supplemented with protected fat in comparison to the control animals. Tyagi *et al.* (2010) also showed significant reduction in the time required for commencement of cyclicity, service period and artificial insemination (AI) per conception with supplementation of 2.5% bypass fat in crossbred cows.

CONCLUSION

From the present investigation it can be concluded that supplementation of bypass fatty acids and *Tinospora cordifolia* enhances the reproductive performance of Murrah buffaloes by increasing the rate of involution and follicular size and reducing the service period. Supplementation also improves conception rate and uterine health by decreasing incidence of post-partum uterine infections.

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