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PHARMACOLOGICAL EFFECT OF DEXAMETHASONE ALONE AND IN COMBINATION WITH METOCLOPRAMIDE ON DIFFERENT PARAMETERS IN LACTATION INDUCED REPEAT BREEDING HEIFERS *VS*. NATURAL LACTATION NULLIPAROUS HEIFERS

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Abstract: The inductions of lactation were carried out in 30 animals, divided into three groups with 10 animals in each group. Group I, animals which were in first lactation (nulliparous) served as control, not given any treatment. Group II, administered with 17β-estradiol at 0.1 mg/kg body weight and progesterone at 0.25 mg/kg body weight were administered from day 1 to 7 twice daily 12 h apart by subcutaneous route at either side neck region on alternate days. Dexamethasone (20mg/animal/day) was administered for 4 days (day 14 to 17) by intramuscular route. The treatment schedule for Group III, from day 1 to 7 were adopted same as group II and Metoclopramide (0.1 mg/kg body weight) and dexamethasone (20mg/animal) were administered for 4 days from day 14 to 17 by intramuscular route. The relative efficacy of lactation induction protocol employed in group II was 90 per cent and while in the group III it was 100 per cent. In both the treatment groups droping of milk from the teats were noticed on day 18 and 19 in four and five animals in group II and III respectively and milking was initiated on day 20. The first day milk was watery, yellowish to white in colour. The milk secretions in all the animals become normal within 3 to 7 days. The animals reached to peak mean daily milk yield of 4.73±0.15, 4.38±0.16 and 5.06±0.20 liters on day 45, 90 and 60, the mean total milk yield for 90 days were 357.83±4.05, 228.87±4.49 and 349.30±12.95 liters and for 305 days were 1224.92±14.78, 753.07±14.09 and 1156.95±33.39 liters in group I, II and III respectively. The mean total milk yield for 90 and 305 days were significantly higher ($P<0.001$) in group I and group III as compared to group II. The mean total milk yield for 90 and 305 days were increased in group I than group III but non significant ($P>0.05$). The peak mean milk fat composition of 5.87 ±0.16, 4.05±0.09 and 4.27±0.10 per cent on day 1, 45 and 90 and the lowest mean SNF composition of 8.18±0.03, 8.55±0.05 and 8.36±0.06 per cent on day 1, 45 and 90 were observed in group I, II and III group respectively.

Keywords: Lactation induction, efficacy, milk yield, 17β-estradiol, progesterone.

INTRODUCTION

The dairy industry has crippling due to many problems like frequent disease outbreak in the herd, nutritional deficiency and repeat breeding. Among which repeat breeding is the major

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reproductive disorder which makes animal infertile, there by causes decline in milk production and productivity of the dairy cattle which causes economic losses to dairy farmers.

The repeat breeder animal is usually defined as a sub fertile animal which has been served three or more times with a fertile bull or inseminated with fertile semen yet fails to conceive and continuially returns to estrus in the absence of any obvious pathological disorder in the genital tract (Purohit, 2008).

The reproductive disorder in animal is troubling because it is often the higher milk producers that are at the greatest risk of failing to conceive in a timely manner, as discussed by (Loeffler *et al*., (1999). (Erb and Martin, 1980).

To overcome all above problems, adopting artificial induction of lactation technology in unproductive animals, udder can be developed by employing combination of hormones and drugs, thus milk can be obtained even without pregnancy (Verma *et al*., 2004). This technology can be used as a profitable management tool to help production ability back into the herd (Magliaro *et al.,* 2004).

The milk obtained following artificial induction lactation procedure did not differ from that after natural lactations following pregnancy (Narendran *et al*., 1979).

In earlier days artificial induction of lactation in dairy cows has been obtained by administration of estrogens and progesterone for 60 to 180 days (Turner *et al.,* 1956; Meites, 1961). The results obtained by these induced lactations were variable and the long term treatments required made the procedures of limited practical value. More recently, Smith and Schanbacher (1973) have proved that injection of 17β-estradiol and progesterone for 7 consecutive days induced mammary gland growth and lactation in the bovine. Milk yields recorded using this procedure approached those measured during natural lactations following pregnancy. However, there were paucity of information regarding comparative study of lactation induction protocols by using dexamethasone alone and its combinations with metoclopramide are very rarely reported (Mohan *et al.,* 2007) and in order to continue improving the success rate, increase in milk yields, eliminate the variability in milk yields between cows and further research in development of reliable cost efficient protocols for induction of lactation is very much needed. Keeping all these facts in view the present study has been taken up to find an alternate way to reduce the economic burden of heavy maintenance costs on farmers. Hence it is proposed to induce lactation in dry or unproductive animals.

MATERIALS AND METHODS

The animals for current study were selected from various parts of Davanagere district of Karnataka state, India. The study was conducted during the period february-2018 to march-2019 Group I (n=10) comprising of eight crossbred HF and two Jercy cattle, which were in first lactation served as control, where as in Group II and III (n=10) consists of repeat breeding eight crossbred HF & two Jercy heifers each.

The reproductive organs of all the animals were examined per rectally prior to the inclusion into the study to rule out the reproductive disorders, if any.

The body weight of the animals were calculated using the formula:

Body weight (in Kg) = $L \times G^2 / 660$

where L (length) – the distance in inches from point of shoulder to the point of rump,

G (Girth) – is the circumference of chest in inches.

The selected animals were dewormed with fenbendazole at the dose of 5 mg/kg body weight ten days prior to initiation of experiment. All the animals were allowed for fifteen days acclimatization period prior to the study.

Administration of drugs

Group I, animals which were in first lactation served as control, not given any treatment. Group II, administered with 17β-estradiol at 0.1 mg/kg body weight and progesterone at 0.25 mg/kg body weight were administered from day 1 to 7 twice daily 12 h apart by subcutaneous route at either side neck region on alternate days. Dexamethasone (20mg/animal/day) were administered for 4 days (day 14 to 17) by intramuscular route.

The treatment schedule for Group III, from day 1 to 7 was adopted as per group II. Metoclopramide (0.1 mg/kg) and dexamethasone (20mg/animal) were administered for 4 days from day 14 to 17 by intramuscular route .

Collection of milk sample

Milk samples were collected from all induced animals regularly for 305 days with the first samples were collected on the first day of milking i.e. day 20 of experiment from all treatment groups. Milk samples were also collected from normally parturated animals regularly from the day of parturition i.e. (control group) on day 1 of parturition to day 305.

The milk were also collected on day 1, 3, 6, 9, 12, 15, 18, 21, 25, 30, 45, and 90 as such without adding any preservatives for analysis of composition such as fat, SNF and CLR, were analysed directly at the time of milk collection by using lactometer available at

SHIMUL, milk union, milk collection centre, Sangahally, Chennagiri taluk, Davanagere district, Karnataka, India.

The data generated for different parameters were tabulated group wise and expressed as mean ± S.E. Two way analysis of variance with Bonferroni post-test for milk yield and one way analysis of variance with Turkey's Multiple comparison test by using Graph Pad Prism trial version 5.00, Graph Pad, Software, San Diego, California, USA.

RESULTS AND DISCUSSION

For comparing the efficacy of the various lactation induction protocols the mean age & body weight of the animals also taken into consideration are depicted in Table 1.

Before conducting the study reproductive organs of all the animals were examined per rectally (depicted in Figure 1) prior to the inclusion into the study to rule out the reproductive disorders, if any. In both the treatment groups (group II and III) lactation were induced successfully by administration of hormones and drugs exogenously. Mammary gland of lactation induced animals showed marked enlargement in size of udder by day 7 is depicted in Figure 2. As the days advances mammary gland became fully distended with fluids and the teats were elongated full and turgid in both the treatment groups dropping of milk from the teats were noticed on day 18 and 19 in four and five animals in group II and III respectively In all the lactation induced animals milking were initiated on day 20 and milking were carried out twice a day in the morning and evening with 12 h interval between the milking. The first day milk was watery, yellowish to white in color. The milk secretions in all the animals become normal within 3 to 7 days.

In the current study the relative efficacy of different treatment protocols employed in the present studies varied between the groups (depicted in Table 2). In group II, out of 10 cows, 9 were responded for the induction therapy resulting 90 per cent success rate with ≥2.5 liters/day of milk. The success rate observed in the current study was in accordance with Hooda *et al*., (1997) and Priscilla *et al.,* (2010), who reported the success rate of 90 percent.

In group III, out of 10 animals all were responded for the induction therapy resulting 100 per cent success rate with ≥ 3.5 liters/day of milk. The success rate in the present study were in accordance with Mohan, (2007) and Mellado *et al.* (2006) who reported similar success rate of 100 per cent. While, the success rate in the present study were higher than the Smith and Schanbacher, (1973); Collier *et al.* (1975); Chakriyarat *et al*. (1978) and Tracy, (2002) who reported the success rate of 60, 69, 82 and 92 per cent respectively. Increased success rate

in the present study might be due to dose, duration and the type of hormones used for the induction of lactation.

The mean daily milk yield was varied between the groups. The mean daily milk yield on day 1 was 1.04±0.07, 0.15±0.01 and 0.35±0.03 liters and reached to the peak mean daily milk yield of 4.73±0.15, 4.38±0.16 and 5.06±0.20 liters on day 45, 90 and 60 in group I, II and III respectively. The mean daily milk yield of group II were significantly decreased ($P<0.001$) on day 1, 3, 6, 10, 12, 15, 20, 30, 45 and 60 become identical on day 75 and 90 as compared to control group. A significantly decrease ($P<0.001$) in mean daily milk yield were noticed in group III on day 1, 3, 6, 10, 12, 15, 20, 30 and 45 and also significant increase ($P<0.01$) on day 60 and 75 and become identical on day 90 as compared to control group presented in Table 3 and Figure 3. Reaching peak daily milk yield in group II was delayed compared to group III. The time taken to reach peak daily milk yield in the current study is in accordance with Hooda *et al.* (1997) and Ramgattie *et al*. (2014), who were reported time taken to reach peak mean daily milk yield varied between 12-14 and 12.7 ± 1.8 weeks. The time taken to reach peak daily milk yield in the present study is higher than the (Smith and Schanbacher, (1973) and Tervit *et al*. (1980) who were reported, time taken to reach peak mean daily milk yields from 7 to 8 weeks on onset of lactation . The time taken to reach peak daily milk yield in the present study is lower than reported by Priscila, *et al.* (2010) who reported delayed daily peak milk production of 15 liters occurred from 104 to 145 days of induced lactation. The delayed peak yield in induced cows might be due to the less amount of secretory tissue present at the starting of lactation as compared with non induced cows, since the mammary development occurs over three week period in the induced lactation and during the last month of gestation (Knight and Wilde, 1993). The variation in time taken to attain peak daily milk yield is suggestive of variation in feeding regimens of the animals, genetic character and health condition.

The mean total milk yield for 90 days were 357.83±4.05, 228.87±4.49 and 349.30±12.95 liter and for 305 days were 1224.92±14.78, 753.07±14.09 and 1156.95±33.39 liters in group I, II and III respectively.

The mean total milk yield were significantly decreased ($P<0.001$) on day 45, 90, 180, 270 and 305 in group II as compared to control group. Where as in group III a significant increase ($P<0.01$) were observed on day 305 and non significant decrease ($P>0.05$) on day 45, 90, 180 and 270 when compared to control group. Also a significant increased ($P<0.001$) in mean

total milk yield were observed in group III on day 45, 90, 180, 270 and 305 as compared to group II presented in Table 4 and Figure 4.

The quantity of total milk obtained in the current study is in accordance with the reported results of Mohan *et al.* (2009), whose study was also conducted in same latitude and longitude. However, higher total milk yield were obtained by Tracy, (2002); Freitas *et al.* (2010); Ramgattie *et al*. (2014). The increased in milk yield obtained by the afore mentioned authors could be due to breed, managemental practices, superior genetic potential for milk yield (Ramgattie *et al*. 2014). Further, Tracy, (2002) had administered recombinant bovine somatotropin once every two weeks on the day of milk let down throughout the period of her experiment (21 weeks) which resulted in higher average daily milk yield.

The animals treated with metoclopramide had a better milk performance, this increase in the milk yield may be because of release of higher concentration of plasma prolactin by metclopramide as suggested by Gupta and Gupta, (1985) and Shridhar and Narayana, (2006).

The mean milk fat composition on day 1 were 5.87±0.16, 1.97±0.14 and 1.30±0.06 per cent and reached to peak mean milk fat composition on day 1, 45 and 90 in control, II and III group respectively. A significant decrease ($P<0.001$) in mean milk fat composition of group II were observed on day 1, 3, 6 , 9, 12 and 15 and become identical on day 18 as compared to control group. Where as in group III significantly decreased ($P<0.001$) on day 1, 3, 6 and 9, become identical on day 15 as compared to control group. However in group III significant decrease ($P<0.001$) in mean milk fat composition were observed on day 1 and 3, non significantly decrease ($P>0.05$) on day 6, 9, 12, 15, 18, 25, 30 and non significantly increase ($P>0.05$) on day 25 and 90 as compared to group II are presented in Table 5 and Figure 5.

The mean SNF composition of milk observed on day 1 were 8.18±0.03, 11.20±0.41 and 12.07±0.13 per cent, gradually decrease and reach to lowest SNF composition of 8.18±0.03, 8.55±0.05 and 8.36±0.06 per cent on day 1, 45 and 90 in group I, II and III respectively. The mean milk SNF composition of group III were significantly increased ($P<0.01$) on day 3 and 12, non significantly increased ($P>0.05$) on day 1, 6, 15, 18, 21 and 30 and non significantly decreased ($P>0.05$) on day 9, 25, 45 and 90 as compared to group II. But the fat composition of milk become identical between control and treatment groups on day from 18 and 15 and SNF on 15 to 25 in group II and III are presented in Table 5 and Figure 6.

In the current study, fat and SNF composition of milk is altered initially in lactation induced cows and become identical within in 15 to 25 days as compared to control group. The current results of attaining milk composition of treatment groups at normal level as compared to

control group were earlier than reported by Ajit *et al*., (2015), who reported normal specific gravity and fat percentage at different evaluation intervals (two to six) in the induced milk suggested it to be safe for consumption after one month of induction. Tervit *et al.* (1980) reported that per cent of milk fat were similar between controls and induced cows. Narendran and Hacker, (1974) examined percentage of fat in milk of lactation-induced cows and lactations following parturition and found no differences in percentage of fat between the two groups. Delouis *et al*. (1978) reported that the milk composition such as fat content of lactation induced cows showed same as found during natural lactations. Linn, (1988) stated that the lactose content of the milk is relatively constant, the content of fat and to a lesser extent protein normally varies widely because of many factors , including genetics, breed, stage of lactation, age, diet composition, nutritional status, environment and season. Barbano and Lynch, (1989); Chalupa and Galligan, (1989); Peel and Bauman, (1987) stated that factors such as stage of lactation, diet, nutritional status affect the fat, total solid and protein content of the milk in an identical manner in bST supplemented and untreated cows.

It is concluded from the current study that repeat breeding animals induced hormonally and treated with a combination of dexamethasone and metoclopramide improves success rates of induced lactations and eliminate the variability in milk yields between cows, but this method of induction of lactation was not successful in increasing milk yields.

Figure 1: Rectal examination by Dr. Murigappa, Professor, Dept. of Veterinary Gynaecology and Obstretics, Veterinary College, Shivamogga

Figure 2: Development of udder after 7 days of hormonal treatment

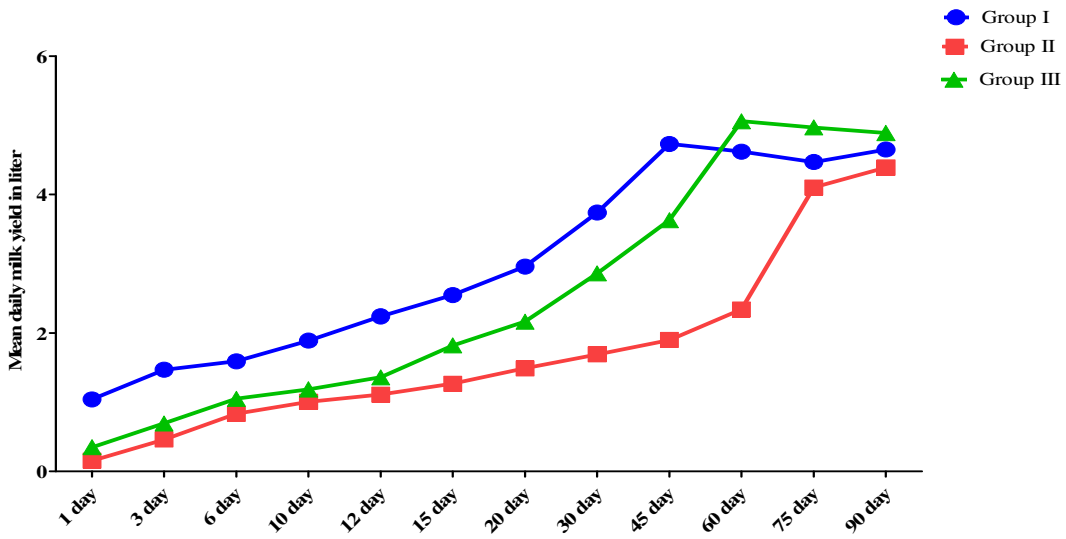

Figure 3 : Comparison of mean daily milk yield (liters) of group I VS. treatment groups

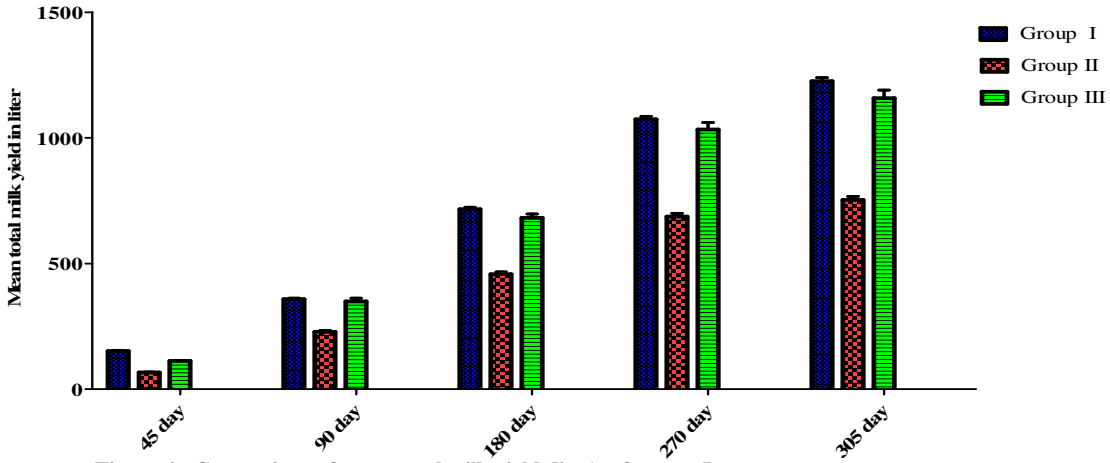

Figure 4 : Comparison of mean total milk yield (liter) of group I vs. treatment groups

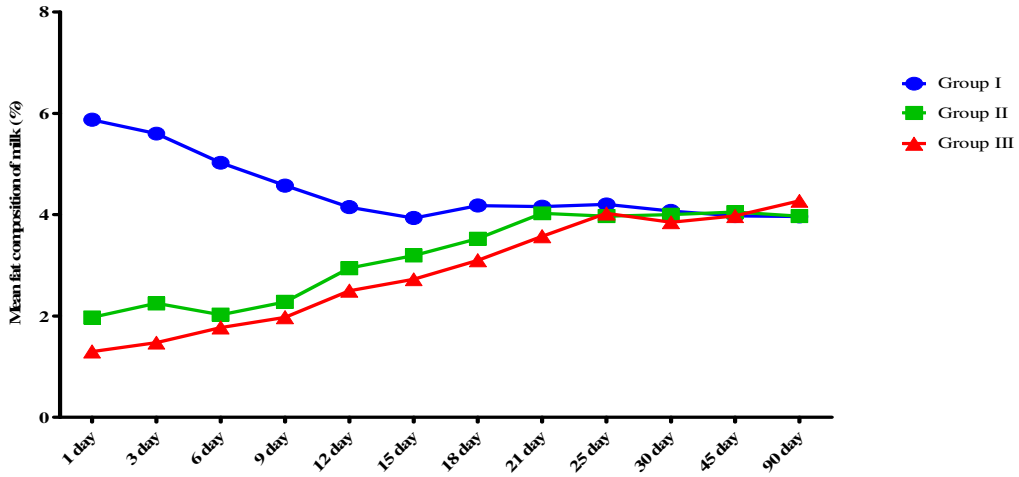

Figure 5: Comparison of mean fat composition of milk (%) of group I VS. treatment groups

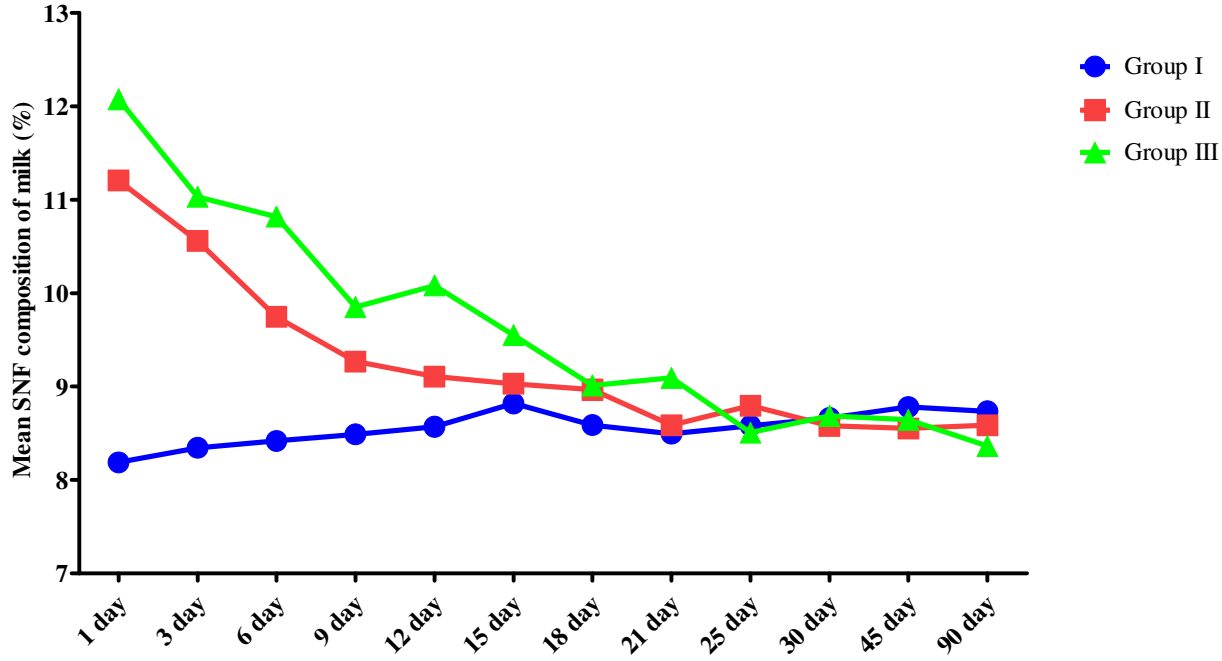

Figure 6: Comparison of mean solid not fat composition (%) of milk of group I vs. treatment groups

Table 1: The mean age and body weight of control and treatment groups

	Group I (n=10)	Group II (n=10)	Group III (n=10)
Age (in months)	45.80±0.57	43.50±081	44.30±0.55
Body weight (in kilogram)	333.50±5.53	310.50±4.24	316.50±6.01

Table 2: Comparative efficacy of treatment protocols in lactation induced groups

	Group II (n=10)	Group III (n=10)
Number of animals responded	9(90)	10(10)
Number of animals not responded	1(10)	0(0)
Total animals	10	10

Table 3: Comparative mean daily milk yield in liters of control and treatment groups

Day	Group I (n=10)	Group II (n=9)	Group III (n=10)
1 day	1.04 ±0.07	0.15±0.01***	0.35±0.03***
3 day	1.47±0.03	0.46±0.04***	0.69±0.04***
6 day	1.59±0.06	0.83±0.04***	1.05±0.05**
10 day	1.89±0.09	1.00±0.04***	1.18±0.07***

12 day	2.24±0.07	1.11±0.05***	1.36±0.09***
15 day	2.55±0.09	1.26±0.06***	1.82±0.06***
20 day	2.96±0.13	1.48±0.03***	2.16±0.11***
30 day	3.74±0.0.17	1.68±0.07***	2.86±0.13***
45 day	4.73±0.15	1.90±0.09***	3.63±0.04***
60 day	4.62±0.14	2.33±0.08***	5.06±0.20*
75 day	4.47±0.15	4.10±0.18	4.97±0.21*
90 day	4.65±0.09	4.38±0.16	4.89±0.27

Values are in Mean ± S. E
Mean values bearing different superscripts within a column are significantly different.
*P< 0.05
**P< 0.01
***P< 0.001

Table 4: Comparative mean total milk yield in liters of control and treatment gropus

Day	45 day	90 day	180 day	270 day	305 day
Group I	151.43 ±3.34	357.83±4.05	715.66±8.11	1073.48±12.15	1224.92±14.78
Group II	66.45±1.28***	228.87±4.49***	457.74±8.99***	686.61±13.49***	753.07±14.09***
Group III	111.67±1.62	349.30±12.95	682.10±14.55	1033.45±27.12	1156.95±33.39**

Values are in Mean ± S. E
Mean values bearing different superscripts within a column are significantly different.
**P< 0.01
***P< 0.001

Table 5: Comparative mean fat and SNF composition of milk in per cent (%) of control and treatment groups

	Mean fat composition of milk			Mean SNF composition of milk		
Day	Group I (n=10)	Group II (n=9)	Group III (n=10)	Group I (n=10)	Group II (n=9)	Group III (n=10)
1 day	5.87 ±0.16	1.97 ±0.14***	1.30 ±0.06***	8.18 ±0.03	11.20±0.41***	12.07 ±0.13***
3 day	5.60±0.23	2.25±0.13***	1.47±0.09***	8.34±0.06	10.55±0.39***	11.03±0.40***
6 day	5.02±0.17	2.02±0.20***	1.77±0.13***	8.41±0.04	9.74±0.26***	10.81±0.37***
9 day	4.57±0.18	2.27±0.09***	1.97±0.13***	8.48±0.08	9.26±0.08	9.85±0.34***
12 day	4.15±0.15	2.94±0.14***	2.50±0.06***	8.56±0.10	9.10±0.10	10.08±0.34

15 day	3.93 ±0.10	3.19 ±0.13**	2.72 ±0.08***	8.81 ±0.07	9.03 ±0.17	9.55 ±0.31
18 day	4.18±0.09	3.52±0.10*	3.10±0.09***	8.58±0.07	8.96±0.12	9.01±0.12
21 day	4.16±0.11	4.02±0.15	3.57±0.11*	8.49±0.12	8.58±0.15	9.09±0.26
25 day	4.20±0.05	3.97±0.15	4.02±0.15	8.58±0.06	8.79±0.11	8.50±0.13
30 day	4.07±0.09	4.00±0.14	3.85±0.15	8.66±0.14	8.57±0.09	8.68±0.14
45 day	3.97±0.10	4.05±0.09	3.97±0.17	8.78±0.09	8.55±0.05	8.64±0.12
90 day	3.96±0.05	3.97±0.11	4.27±0.10	8.73±0.09	8.58±0.04	8.36±0.06

Values are in Mean ± S. E
Mean values bearing different superscripts within a column are significantly different.
*P< 0.05
**P< 0.01
***P< 0.001

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