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Lactogenic efficacy of *Ricinus communis* L. 3X and Calcarea carbonica 3X in Jersey crossbred (JX) cows

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In order to evaluate the lactogenic effect of *Ricinus communis* L. and Calcarea carbonica in Jersey crossbred cows, a study was conducted at Post Graduate Research Institute in Animal Sciences (PGRIAS), Kattupakkam, Tamil Nadu Veterinary and Animal Sciences University, Chennai. Eighteen apparently healthy, pluriparous, Jersey crossbred cows within 3 months post-partum were selected for the study and randomly allotted into three groups as Group I (Control), Group II (5 mL of *R. communis* L.3X P/Oper day for 10 days) and Group III (5 mL of Calcarea carbonica 3X P/Oper day for 10 days). All the selected crossbred cows were maintained under uniform managerial conditions. There was no significant influence ($p>0.05$) observed in feeding, watering habits, physical and behavioral changes in all the crossbred cows. The mean rectal temperature, pulse rate and respiratory rate during different phases of treatment regimens did not significantly differ ($p>0.05$) in all the groups. The status of conjunctival mucus membrane and vaginal mucus membrane did not show any significant variations ($p>0.05$) during the entire period of study. The fat, solid not fat and total solid percentage in milk were significantly ($p<0.01$) increased in cows treated with *R. communis* L. 3X than the cows treated with Calcarea carbonica 3X. The results demonstrated the potent galactogogue activity of *R. communis* L. 3X in yielding a good milk promoting response and favorable milk composition which is essential to improve the economic status of the agrarian community. The pharmacodynamic and pharmacokinetic action of the drug needs to be characterized by further studies.

Keywords: Calcarea carbonica, Crossbred cows, Galactogogue, Homeopathy, *Ricinus communis*

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The nourishment of the neonate is facilitated by the secretion by the dam of an opaque, white fluid called milk which is rich in nutrients such as proteins, fats and essential vitamins[1,2]. The consumption of milk from bovine and non-bovine sources has been prevalent in humans since prehistoric times[3]. Hence, dairy production is of vital importance to livestock owners because it has direct influence on cost and returns. In this context, agalactia or hypogalactia, a form of lactation failure severely affects optimal milk production in cows. This is alleviated by the use of certain natural and/or synthetic substances called galactogogues[4]. Lactation in cows is influenced by several factors such as physiological state, nutritional intake and environmental factors, all of which affect the quality and quantity of milk production. Natural galactogogues based on herbal origins are favored over their synthetic versions as they do not show any adverse side effects in cattle and do not leave residues in tissues, secretions and excretion of milk[5,6]. Of the most widely used natural galactogogues, extracts from *Ricinus communis* L. have been used extensively in the form of topical applications and oral formulations in traditional medicinal practices such as Ayurveda, Homeopathy and Unani[7-9]. The *Warli* tribes of Dahanu, Maharashtra have been traditionally using the leaves of *R. communis* L. where the nursing mothers apply the heated leaves to their breasts to facilitate increased milk flow and lactogenesis[10]. Likewise, the decoction of fresh leaves and seed oil of *R. communis* L. are applied traditionally as a massage on breasts to improve lactation in several tribal groups in India[11].

Similarly, one of the critical nutrients influencing dairy production in cows is Calcium (Ca) as it has been reported that a deficiency of dietary calcium

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causes an immediate reduction in milk production in lactating cows[12]. Calcarea carbonica, commonly referred to as Calcarea, is a homoeopathic remedy made from calcium carbonate (found in the middle layer of oyster shells a substance of carbonate of lime) has long been prized for its ability to treat a wide range of chronic ailments, especially those that affect the metabolism, bones, and joints. Calcarea carbonica is also used in homeopathy to treat symptoms like tender breasts, mastitis, hyperlactation and agalactia in affected women[13].

Based on this, the present study was formulated with an objective to evaluate the lactogenic effect of *R. communis* L. and Calcarea carbonica in improving lactation in Jersey crossbred cows (JX) and to elucidate changes in milk composition following the administration of these galactogogue homeopathic drugs.

Materials and Methods

Study design and animal maintenance

The study was conducted at Post Graduate Research Institute in Animal Sciences (PGRIAS), Kattupakkam, Tamil Nadu Veterinary and Animal Sciences University, Chennai. The study was conducted from March 2017 to March 2018. During the study, 3 months adaptation period was given before start of the trial. Eighteen apparently healthy, pluriparous, Jersey crossbred cows of second calving parturition within 3 months were selected. The animals were maintained under uniform managerial conditions were utilized for this study. The selected crossbred cows were randomly allotted into three groups as Group I (n=6) Control, Group II (n=6) treated with 5 mL of *R. communis* L. 3X per day orally for 10 days and Group III (n=6) treated with 5 mL of Calcarea carbonica 3X per day orally for 10 days, respectively. The dosage was determined according to the weight of the cows. Each crossbred cow with milk yielding up to 5 L per day was fed with 25 kg of green fodder, 5 kg of roughage and 2 kg of concentrate mixture per day. Those cows yielding more than 5 L milk were additionally provided with ½ kg of concentrate mixture for every 1 L of milk over 5 L.

Preparation of galactogogue formulation

The galactogogue formulation of *R. communis* L. 3X and Calcarea carbonica 3X were prepared according to the method described by Boericke, 2007[13] and Homoeopathic Pharmacopoeia of India, Ministry of Health, 1983[14]. Several minerals are found in *R. communis*, such as Calcium (27,433±120 µg/g), Iron (254±18 µg/g), Potassium (19,490±223 µg/g) and Calcarea carbonica is rich in calcium (~98%) which facilitate their role in inducing the good quality of milk production[15].

Evaluation of feeding, watering and behavioral responses

The feeding and watering habits of all the groups were monitored and recorded between day 0 to 20 immediately before and after administration of drugs from the beginning of treatment regimen. The physical and behavioral changes constituting estrus behaviors such as mounting, bellowing, reduced feed intake were studied according to the method described by Rao and Rao (1981)[16] and Krishnakumar (2001)[17] with slight modification and continuously monitored and recorded for 20 days from the initiation of treatment.

Evaluation of vital signs

The vital signs were recorded at 3 pm daily in all the groups before milking up to 20 days from the initiation of treatment. The body temperature of cattle was determined by inserting thermometer into the rectum and touching the mucus membrane or inner lining of the rectum for 1 min on a daily basis between days 0 to 20 from the initiation of treatment. The pre, peri and post treatment mean (±SE) rectal temperature were also recorded. The pulse rate was recorded daily between days 0 to 20 from the initiation of the treatment via the coccygeal artery felt below the base in the centre of tail. The pre, peri and post treatment mean (±SE) pulse rate were also recorded. The respiratory rate was recorded daily between days 0 to 20 from the initiation of the treatment. The pre, peri and post treatment mean (± SE) respiratory rate were also recorded.

Evaluation of galactogogue activity

All the control and treated crossbred cows were milked twice a day at 3 am and 3 pm following drug administration and the quantity of milk let down were recorded between days 0 to 20 from the beginning of treatment. The fat percentages were estimated as per the Gerber method[18]. Milk samples were collected on days 0, 5, 10, 15 and 20 in all the groups. The fat percentages in milk at pre, peri and post treatment phases in all the treatment regimens were also determined. The Solid-Not-Fat (SNF) content of the milk between days 0 to 20 from the beginning of treatment were determined by subtracting the fat percentage from total solid percentage as per the Gerber method[18]. Further, pre, peri and post solid not

fat content was also recorded in all the groups. Total solid (TS) was calculated as the sum of fat and Solid Not Fat per cent per the Gerber method[18]. The total solid in milk were calculated between days 0 to 20 from the beginning of treatment and at pre, peri and post treatment phases in all the groups.

Statistical analysis

The statistical analysis was performed as per the method described by Richardson (1985)[18] and Snedecor and Cochran (1989)[19] using GraphPad Prism (version 8.0.2 (263); GraphPad Software Inc., La Jolla, CA, USA)[20]. Statistical significance was set at 5% probability level.

Results

Feeding, watering habits, physical and behavioral changes

There was no significant difference observed in physical and behavioural changes in all the groups at pre, peri and post treatment periods. However, it was observed that one cow (17%) in groups I and II and two cows (33%) in group III, showed physical and behavioral changes on days four, eight and ten (peri treatment period) and on day eleven (post treatment period), respectively. The results showed that the administration of *R. communis* L. 3X and Calcarea carbonica 3X had no influence statistically (p>0.05) on feeding, watering habits, physical and behavioral changes in crossbred cows.

Vital signs

***Mean rectal temperature* (°C)**

The mean rectal temperature (°C) recorded in the present study ranged from 38.68 to 39.10°C in group I;38.63 to 39.20°C in group II and 38.70 to 39.93°C in group III between days 0 to 20. The mean rectal temperature (°C) recorded in the present study were found to be non-significant (p>0.05) between all the groups between days 0 to 20 from the beginning of treatment (Fig. 1).

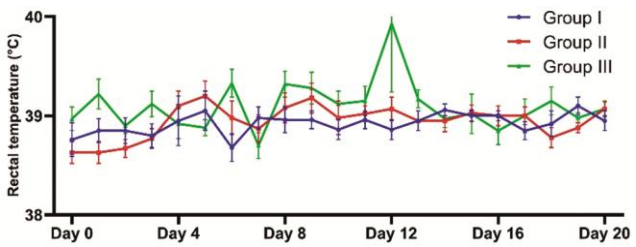

Fig. 1 — Mean (± SE) rectal temperature (°C) with different treatment regimens in crossbred cows

Pulse rate per minute

The pulse rate per minute recorded in the present study ranged from 67.67 to 70.17 per min in group I, 76.33 to 79.67 per min in group II and 87.00 to 89.00 per min in group III between days 0 to 20. The pulse rate per min recorded in the present study had no significant difference (p>0.05) in all the groups between days 0 to 20 from the beginning of treatment (Fig. 2).

Respiratory rate per minute

The respiratory rate per minute in the present study was recorded as 31.20 to 32.40 per minute in group I, 31.10 to 33.80 per minute in group II and 34.80 to 38.85 per minute in group III. The respiratory rate per minute recorded in the present study had no significant difference (p>0.05) in all the groups (Fig. 3).

Conjunctival mucus membrane and vaginal mucous membrane

The status of conjunctival mucus membrane and vaginal mucus membrane were calculated with Chi square test (2x2 contingency). In the present study, all the crossbred cows (100%) had pink and moist conjunctival mucus membrane at pre, peri and post treatment period which revealed there was no significant difference (p>0.05) in the status of conjunctival mucus membrane observed in all the groups. The changes in vaginal mucus membrane were recorded in one of two categories as pink and moist and congested or hyperemic. There was no statistically significant difference (p>0.05) in the pre-treatment phase as the vaginal mucus membrane was

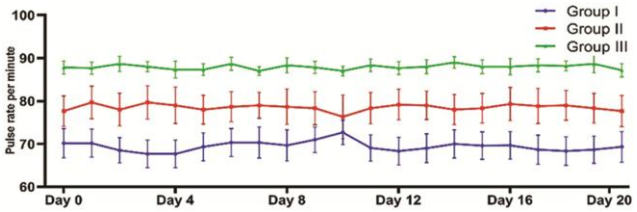

Fig. 2 — Mean (±SE) Pulse rate per min with different treatment regimens in crossbred cows

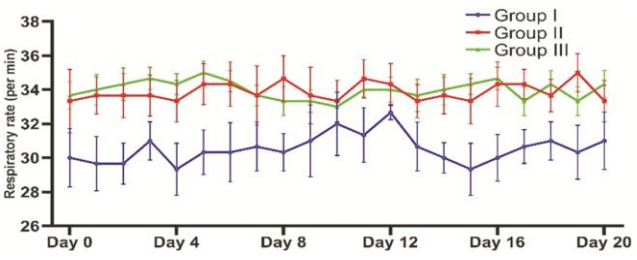

Fig. 3 — Mean (±SE) respiratory rate per min with different treatment regimens in crossbred cows

pink and moist (100%) in all the groups. However, statistically significant ($p<0.05$) difference was observed between groups I and III while no statistically significant difference ($p>0.05$) found between groups I and II at peri treatment period and post treatment period, respectively.

Milk yield

The quantity of milk yield (mean ± SE) with different treatment regimens of crossbred cows revealed that there was no significant difference ($p>0.05$) on day 0 between all the groups. However, between days 2 and 15, the milk yield was significantly higher ($p<0.01$) in groups II and III than group I, although group III had significantly ($p<0.05$) lower milk yield than group II. Similarly, between days 15 and 20, the milk yield was significantly higher ($p<0.01$) in group II than groups I and III crossbred cows (Fig. 4).

The mean (±SE) milk yield at pre, peri and post treatment phases with different treatment regimens in crossbred cows were recorded as 5.16±0.2, 7.39±0.25 and 6.43±0.02 L, respectively in group II and 5.55±0.18, 5.79±0.16 and 5.07±0.09 L, respectively in group III crossbred cows (Fig. 5).

Fat concentration

The milk fat percentage ranged from 3.58±0.21 to 3.72±0.18% in group I, 4.70±0.35 to 5.07±0.31% in group II and 3.67±0.17 to 4.48±0.04% in group III crossbred cows, respectively. In the present study, group II cows had significantly higher ($p<0.01$) fat percentage than group I and III cows on days 10, 15 and 20 (Fig. 6). The fat percentage recorded at pre, peri and post treatment period in the present study was 3.72±0.12, 4.97±0.20 and 4.93±0.12%, respectively in group II and 3.67±0.17, 4.38±0.21 and 3.85±0.15%, respectively in group III crossbred cows. The fat percentage in group II crossbred cows were significantly higher ($p<0.01$) than group III crossbred cows.

Solid Not Fat (SNF)

The mean Solid Not Fat (SNF) concentration in milk ranged from 8.23 to 8.34%; 8.27 to 8.91% and 8.24 to 8.71% in groups I, II and III crossbred cows, respectively. The values of SNF observed in the present study revealed that the solid not fat content was significantly higher ($p<0.01$) in group II cows than group I and III cows on days 10 to15 (Fig. 7). The analysis revealed that there was no significant difference ($p>0.05$) at pretreatment phase in all the groups. Group II cows had significantly higher ($p<0.01$) solid not fat percentage than groups I and III cows at peri and post treatment phases; however, there was no significant difference ($p>0.05$) between groups I and III at peri and post treatment phases.

Total solids

The mean total solid concentration in milk ranged from 12.01 to 12.64%; 12.03 to 14.05% in and 11.91

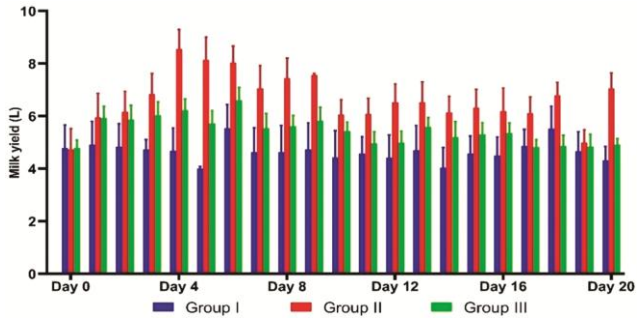

Fig. 4 — Mean ± (SE) milk yield with different treatment regimens in crossbred cows

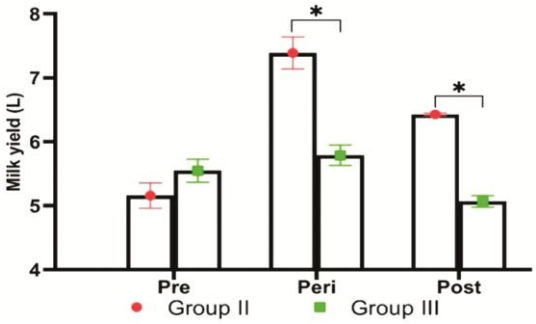

Fig. 5 — Milk yield (L) at pre, peri and post treatment phases with different treatment regimens in crossbred cows (*p<0.01)

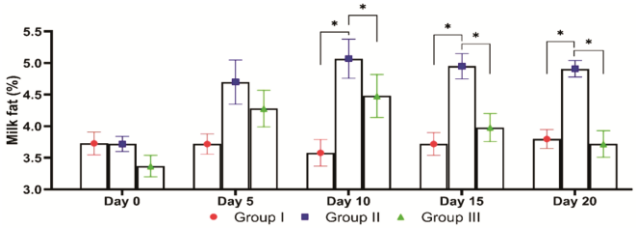

Fig. 6 — Mean (± SE) milk fat percentage (%) with different treatment regimensin crossbred cows (*p<0.01)

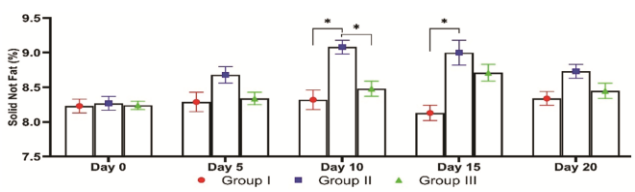

Fig.7 — Mean (± SE)Solid Not Fat (SNF) percentage (%) with different treatment regimensin crossbred cows (*p<0.01)

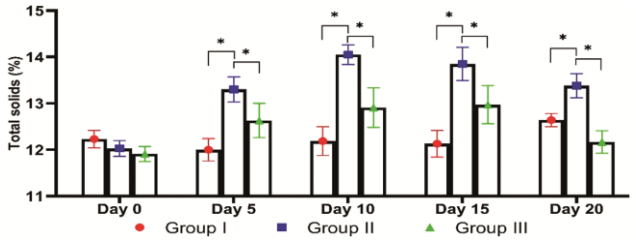

Fig. 8 — Mean (± SE) Total Solid (TS) percentage with different treatment regimens in crossbred cows (*p<0.01)

to 12.97% in groups I, II and III crossbred cows, respectively. The total solid percentage was significantly higher (p<0.01) in group II cows than group I and III crossbred cows (Fig. 8). The analysis revealed that there was no significant difference (p>0.05) at pretreatment phase between all the groups. Group II cows had significantly higher (p<0.01) total solid percentage than group III cows at peri and post treatment phases.

Discussion

The deviation of physical and behavioral changes and reduced feed and water intake observed in the present study in group II cows (treated with 5 mL of *R. communis* L. 3X) and group III cows (treated with 5 mL of Calcarea carbonica 3X) might be due to expression of the estrus signs by the individual crossbred cow at the time of estrus which concurred with the observations of Rao and Rao (1981)[16] where such deviations are the common signs of estrus. With regards to the vital signs, the rectal temperature was found to be consistent with earlier studies[21,22] in which the rectal temperature ranged from 38.93 to 39.4°C in lactating dairy cows. However, the crossbred cows in group II had slightly elevated temperature than 39°C during few days after treatment which might be due to increase in metabolic rate during periods of lactation[23]. Although the results for the pulse rate obtained were in accordance with the observed range of 70 to 77 per minute in crossbred cows[24], it is in contrast to the results obtained in other studies[25] wherein the mean pulse rate ranged between 59.79 to 63.56 per minute in crossbred cows. The results for the respiratory rate were also consistent with the rates determined earlier by Kumar[26], but found to be higher in comparison with rates recorded by Naik *et al*.[22] where the mean respiratory rate of Punganur cattle and bulls ranged from 22.52 to 27.98 per minute. Variations in the season, breed, lactation and stress factors such as drug administration mediated action of phytoestrogens might have led to increased pulse and respiratory rates[25-27] in the present study.

The conjunctival mucus membrane is the membrane that lines the inside of the eye lids and the third eye lid which covers the outside of the sclera[28]. The results obtained in the present study revealed that the galactogogues administered to improve milk production did not produce any side effects in the crossbred cows and were in agreement with the findings of Stockman[29] where the normal and healthy cattle have a pair of clear, bright, alert eyes with clear appearance. While there was no significant difference observed in the appearance of the vaginal mucus membrane during the pre-treatment period in all the groups, the occurrence of congested or hyperemic vaginal mucus membrane during the peri and post treatment periods in groups II and III crossbred cows compared with group I might be due to elevated estrogen and lower progesterone concentrations in circulation at the time of estrus leading to an increased blood flow to the reproductive organ resulting in the congested or hyperemic vaginal mucus membrane[30].

The administration of *R. communis* L. 3X and Calcarea carbonica 3X had led to an increased milk production than the control group in the present study which are comparable with the results of Bhatt *et al*.[31] in that the supplementation of Ruchamax led to an improvement of milk production in cows. The results obtained in the present study was concurred with the observations of Jeyakumar *et al*.[32] wherein the daily milk yield recorded was 4.95 L in control and 5.43 L in calcium administered cows. The increased milk yield in group II cows than in groups I and III crossbred cows might be due to the stimulation of the dopamine receptors through the hypothalamus and pituitary glands leading to prolactin secretion[33] following the administration of *R. communis* L. 3X. However, further studies are warranted to determine the mechanism of action. The results observed in the present study for an increase in fat concentration might be due to conversion of lipids into triglycerides as beta oxidation of fat in the digestive tract[34] and were consistent with earlier studies[35,36] where the fat content in cow milk was recorded as ranging from 3.80 to 4.48% along with increase in milk production. Further, the administered galactogogue might be involved to increase the ruminal activity as calcium propionate supplementation had increased rumen

microbiota and fermentation thereby stimulating enhanced production of total volatile fatty acids (VFA), acetate, propionate and valerate in rumen[37,38]. Hence, further study is warranted to identify the ruminal environment status which should be correlated with fatty acids and fat concentration.

In the present study, the variation in solid not fat concentration might be due to climate, breed, managemental factors, the administration of galactogogue drugs. After administration of galactogogue drugs as *R. communis* L. 3X and Calcarea carbonica 3X, the solid not fat content was elevated at peri and post treatment periods which were found to concur with the results of earlier studies[39,40], where the results indicated that the solid not fat ranged from 8.29 to 8.99% in crossbred cows. In the present study, total solid percentage concurred with the results of Yogi *et al.*[40] and Mirzadeh *et al.*[41] but differed from Babu Rao and Jaya Ramakrishna (1983)[42] and Kaushik and Tandon[43]. These variations might be due to the time of milk collection at morning or evening, breed character and stage of lactation, season and the type of galactogogue drugs used. The improvement of total solid content in milk in the present study following galactogogue administration might be due to the presence of trace minerals like iron, calcium and potassium which promotes milk production[44].

Conclusion

The results from the present study showed that the administration of *R. communis* L. 3X was found to be very effective in improving milk production along with fat, solid not fat and total solid contents. Hence, it is recommended that the administration of *R. communis* L. 3X is essential not only to improve milk production but also to enhance the dairy economy of the agrarian community. However, further studies are warranted to elucidate the characterization of pharmacodynamic and pharmacokinetic actions of the drug.

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Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

The Authors MV and KK conceived and designed the study. Data collection and experimentation done by MV under the guidance of KK. All authors analyzed the data and KK critically revised the article. BSS helped in the preparation of the figures and the manuscript. All authors contributed to editing and reviewing of the manuscript. All authors approved the manuscript.

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Data Availability

The data collected in the study have been presented completely in the article.

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