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Effects of supplementation of poly-herbal mixture on immunoglobulin concentrations and somatic cell count in colostrum of Karan Fries cows

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Abstract
The present study was conducted to assess the efficacy of supplementation of poly-herbal mixture on immunoglobulin concentrations and somatic cell counts in the colostrum. Fourteen Karan Fries cows in their late gestation were selected and randomly divided into 2 groups. Cows in one group were supplemented with a mixture containing herbs and potash alum from 30 days antepartum to 30 days postpartum. *Trachyspermum ammi* (Ajwain), *Asparagus racemosus* (Satawar), *Ocimum sanctum* (Tulsi), *Emblica officinalis* (Aamla), *Cinnamomum zeylancium* (Dalchini) and potash alum were used for preparation of the mixture. Total immunoglobulin concentrations in colostrum were determined on the first, third, and fifth days of lactation using ELISA method. Total somatic cell counts were also estimated in colostrum on the same days. Results showed that supplementation of mixture significantly ($P<0.05$) enhanced the total immunoglobulin in the colostrum whereas at the day of calving there were no significant difference in SCCs between the two group. But at 5th days of calving, colostrum SCCs was significantly lower in treatment group as compared to control group. The study clearly indicated that supplementation of poly-herbal mixture enhanced immunoglobulin concentrations as well as reduced the somatic cell counts in colostrum.

Keywords: Karan Fries cows, Immunoglobulin concentrations, Somatic cell counts, Polyherbal mixture

Introduction
Cows play vital role in milk production for human consumption among the livestock. They contribute greatly to the agricultural economy throughout the world (Gogaev *et al*., 2019) [7]. The importance of cow production efficiency is increasing throughout the world to meet the increasing demands of the human population for milk. High demand for milk encourages dairy farmers to keep yielding cows. High yielding cows are usually facing production stress, which cannot be solely fulfilled by the feed they take. So, modern high-yielding dairy cows enter a state of negative energy balance (NEB) around calving when the energy demand for maintenance and lactation exceeds that of dietary energy intake (Bauman & Currie 1980) [3]. Due to a negative energy balance High-yielding dairy cows experience a series of physiological as well as metabolic changes during the last stage of gestation. It leads to oxidative stress, which is a common characteristic of the transition period (Abuelo *et al*., 2015) [1]. This stress impairs the functional capabilities of immune cell populations, including the lymphocytes responsible for immunoglobulin synthesis (Lacetera *et al*., 2005; Sordillo and Aitken, 2009; Cuervo *et al*., 2021) [9, 14, 6]. Immune dysfunction in dairy cattle further results in infectious diseases such as mastitis during the post-partum stage (Barkema *et al*., 2009) [2]. During this period, it is essential to reduce stressors and reactive oxygen species production to potentiate immunity. Several studies have been conducted to prove the relationship between cow oxidant status during the transition period and colostrum quality and, subsequently, the incidence of disease postpartum. These studies suggested that the higher the producing cow, the greater the oxidative stress and, subsequently, the greater the chances of disease postpartum. A herbal approach to combating this stress is a noble one. Much research has been done to prove the in-vitro antioxidant potential of herbs. Bioactive compounds (e.g. thymol, cinnamaldehyde, cineol, methyl eugenol, anethol, allicin, etc.) present in herbs have been used as immunomodulators to prevent diseases, enhance performance in stress-related syndromes,

and increase resistance against infection. Kuri *et al*., (2019) [8] have observed that supplementation with a polyherbal mixture improved the performance of buffalo and cows respectively. They are a side effect-free alternative to dealing with this problem. Herbs also enhance immunity. Keeping in view the aforementioned fact, this study was designed to investigate the effect of herbs on postpartum colostrum quality as well as immunoglobulin concentration in it. For this study, the selection of herbs with greater antioxidant properties was based on the literature.

Materials and Methods

The experiment was conducted at the Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana. It is located at 29.42°N (latitude) and 79.54°E (longitude) at an altitude of 250 m above the mean sea level in the bed of the Trans-Gangetic alluvial plain. The soil texture was sandy loam. The average annual maximum and minimum ambient temperature ranges between 45 °C and 1.4 °C. The mean annual relative humidity ranges between 41% and 85%, and the annual rainfall of this area ranges from 760 to 960 mm. The experiment was carried out from September to December. During the study period, mean environmental minimum temperatures (°C) was 19.15±0.39, maximum temperature (°C) was 31.20±0.48 and relative humidity (%) was 69.36±5.54. At the beginning of the experiment dried herbs *viz*. Trachyspermum ammi, Asparagus racemosus, Ocimum sanctum, Emblica officinalis, Cinnamomum zeylancium and potash alum were stored in a cool and dry place. These were pulverized and mixed. The mixture was prepared by mixing each pulverised ingredient in a specific proportion (Table 1). Fourteen crossbred (Karan-Fries; Holstein-Friesian ×Tharparkar) cows were selected at 30 days before the expected date of calving from the Institute's experimental herd. The experimental cows had an average initial body weight of 503.37±7.32 kg. They were randomly divided into two groups of seven cows each based on parity (2-5), BCS (2.5-3.5) and 305-day milk yield (4679.36 ± 256.84) kg. Both groups were fed as per NRC 2001. Group I, without any supplementation, acted as control. Cows in group II were supplemented individually with polyherbal-potash alum mixture that acted as treatment. At the start of experiment (30 days ante-partum) cows were weighed and they were subjected to a polyherbal mixture as per their average body weight. Feeding was done as a top dressing on a concentrate mixture in the morning up to 30 days postpartum.

Table 1: Composition of Polyherbal mixture.

Herbs	Amount (mg/kg B.wt.)
Trachyspermum ammi	50
Asparagus racemosus	100
Ocimum sanctum	50
Emblica officinalis	50
Cinnamomum zeylancium	50
Potash Alum	10

To assess the immunoglobulin concentration, a 50-ml colostrum sample was collected at 1st, 3rd, and 5th days postpartum in a clean reagent bottle. Immediately after collection, the samples were transported to the laboratory on ice for further processing. For the processing of colostrum A 20-ml colostrum sample was taken in a 100-ml glass beaker, heated in a water bath to 37 °C, and then 0.5 ml of 0.5% Rennet solution (250 mg Rennet in 50 ml distilled water) was added. After 10 minutes, the clotted sample was mixed by a glass rod and filtered through Whatman No. 42 filter paper overnight. Ig levels were estimated by enzyme-linked immunosorbent assay (ELISA) using a kit from MyBioSources. This sample was stored at -4 °C and further analyzed.

Somatic cell counts (SCC) in fresh milk was counted by the microscopic method. The SCCs of the milk sample was measured microscopically at 40X in 200 fields, and the average number of cells per field was multiplied by the microscopic factor (8.81134633). The microscopic factor was determined by using an ocular and a stage micrometer.

Somatic cell count/ml of milk = average cell count in one field x 8.81134633

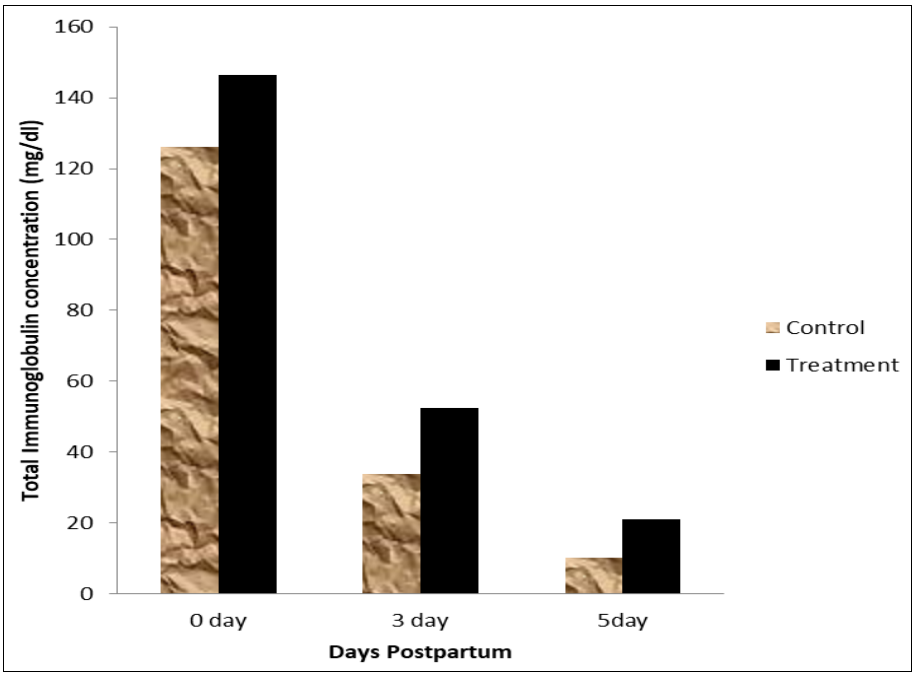
All analysis was done using Sigma Plot version 11 software. Data were analyzed statistically by one way ANOVA with interaction to compare the effect of supplementation. Data are presented as mean ± SE. The level of statistical significance was set at $p<0.05$.

Results and Discussion

The present study was done to estimate the influence of a polyherbal mixture on immunoglobulin concentration in colostrum and the somatic cell count of colostrum in dairy cows. The results of this experiment are presented in Table 2 and Graph 1. On the day of calving, the total immunoglobulin concentration in colostrum was significantly higher ($p<0.05$) in treatment as compared to control. On day 3, colostrum total immunoglobulin was again significantly ($p<0.05$) high in the treatment group as compared to the control. At day 5, postpartum, total immunoglobulin was significantly higher in treatment. Higher concentrations of immunoglobulin increase the chances of survival for calf-borne infections in these cows. It is largely responsible for the production of antibodies and lymphocytes in calves (Riedel-Caspari and Schmidt, 1991a) [10]. A similar result was also reported by Kumar *et al*. (2014 when supplemented with Asparagus at 100 mg/kg body weight for 60 days ante-partum. Sahoo *et al*. (2001) [11] also evaluated the effect of supplementation of polyherbal immunomodulators (Immu-21) containing *Ocimum sanctum*, *Emblica officinalis*, *Withania somnifera*, and *Tinospora cordifolia* on black Bengal goats in the last month of pregnancy and reported a significant ($p<0.05$) increase in colostrum immunoglobulin.

Table 2: Total immunoglobulin concentration in colostrum (mg/ml) in different group of cows (Means ± SE)

Groups	1st Day	3rd Day	5th Day
Control	126.17 ± 5.41^{Ba}	33.86 ± 2.27^{Bb}	10.11 ± 2.66^{Cc}
Treatment	146.42 ± 0.97^{Aa}	52.32 ± 1.35^{Ab}	20.91 ± 3.18^{Ac}



Graph 1: Total immunoglobulin concentration in colostrum

The somatic cell count actually depicts the udder's health status postpartum. The results of colostrum somatic cell counts for these two groups have been presented in Table 3. Colostrum SCCs were about 2.44 x 10^5 per ml in the colostrum of the control group on the day of calving. No significant (p<0.05) difference was observed in colostrum somatic cell count between the groups on the day of calving. The somatic cell counts decreased significantly at day 3 of early lactation in all the groups. On day 5 of early lactation, SCCs were significantly lower in the treatment group as compared to the control group. A reduction in somatic cell count can be correlated with the immune-modulatory, anti-inflammatory, and antithrombotic properties of *Trachyspermum ammi* (Bonjar, 2004) [4]. In another study, Chandra *et al*., (2017) [5] reported that supplementation of a poly-herbal mixture containing herbs *viz*. *Foeniculum vulgare*, *Trachyspermum ammi*, *Trigonella foenumgraecum*, *Zingiber officinale*, *Anethum graveolens*, and *Elettaria cardamomum* to periparturient buffaloes significantly (P<0.05) reduced the somatic cell by 23.76% as compared to control. Singh *et al*., (2019) [13] also reported that polyherbal mixture containing *Azardirachta indica*, *Boswellia serrata*, *Vitex nigundo*, *Ocimum sanctum*, and *Tinospora cordifolia* was found to improve the somatic cell count and milk resistance profile in dairy cows with subclinical mastitis. Similar results were reported by Sharma *et al*. (2014) [12]. These results are probably the outcome of the anti-inflammatory properties of the supplement.

Table 3: Somatic cell counts x 105/ml in colostrum and milk of different group of cows (Means ± SE)

Groups	Days early lactation		
	0	3	5
Control	2.44±0.08Aa	1.84±0.11Ade	1.81 ± 0.13^{Acde}
Treatment	2.40±0.15Aa	$1.77\pm0.13^{A}b$	1.62 ± 0.11^{Bcd}

Conclusion

Early detection of stress conditions and their impact on the production performance of cows is necessary to strategize dairy farm operations. Somatic cell counts are one of the most important tools to detect cows with subclinical mastitis. The results of the present study depict that supplementation with a poly-herbal mixture is an effective strategy to enhance immunoglobulin production as well as reduce somatic cell counts. A decrease in somatic cell counts indicates that the poly-herbal mixture is boosting mammary health as well. It can be applied as a suitable alternative for improving the nonspecific immune system of periparturient cows. Further experiments are required on the dose-response relationship, formulation, and standardization to substantiate a more promising recommendation.

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