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RESEARCH ARTICLE

Effect of supplementation of phyto-pharmaceutical product on the health and productivity of crossbred cows during transition period

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Abstract: The present study was conducted to investigate the efficacy of supplementation of phyto-pharmaceutical product on the immune status of cows during transition period. Twelve crossbred cows in their late gestation were selected and randomly divided into two groups as control group (CON) and supplemented group (SG). The cows in the CON were fed as per the NRC standards while SG were supplemented additionally with 20g of poly-herbal formulation containing *Boswellia serrata* (Burseraceae) and 20g of *Berginia ciliata* (Saxifragaceae) for 21 days prepartum to 7 days postpartum. Immune status *viz*., total leucocyte count (TLC), neutrophil percentage (%), lymphocyte percentage (%), neutrophil: lymphocyte ratio (N:L), *in-vitro* phagocytic activity (PA) of blood neutrophils, plasma cortisol level, interleukin-2 (IL-2) and interleukin-8 (IL-8) were estimated as well as effects were studied on milk for somatic cell count (SCC) and its constituents (fat, protein and lactose) too. Results showed that supplementation mixture significantly ($P<0.05$) reduced cortisol levels, IL-2, IL-8, TLC, neutrophil % and neutrophil: lymphocyte ratio whereas lymphocyte % increased. PA also increased significantly ($P<0.05$) on day of parturition. Milk SCC levels were higher ($P<0.05$) and milk lactose was lower ($P<0.05$) in the CON as compared to the SG. It was inferred that feeding of the phyto-pharmaceutical product effectively reduces stress and enhance immunity during transition period.

Keywords: Crossbred cows, Poly-herbal formulation, immune status, Phagocytic activity, Transition period

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Introduction

The metabolism of cow shifts from pregnancy to lactation during transition period. Reactive oxygen species (ROS) results due to the physiological and biochemical reactions because of this shift (Sordillo, 2009). Immune cells are most sensitive to ROS production, because their membranes contain higher concentration of poly-unsaturated fatty acids which are very susceptible to peroxidation. During the periparturient period animals are more prone to the infections and diseases due to stress and several physiological changes that have been well treated by antibiotics. Although antibiotic use have been an essential part of disease control but it adversely affected the stimulation of growth and influenced the prevalence of resistance in animal bacteria (Newman, 2002). In evidence of high disease incidences, development of antibiotic resistance, drug residue in milk and heavy economic losses worldwide. Herbal feed additives are a very good alternative to antibiotics and they act by affecting the feeding pattern or effect the growth of microorganisms in the rumen, or stimulate the secretion of different digestive enzymes, which in turn may improve the efficiency of nutrients utilization or stimulate the milk secreting tissue in the mammary glands, resulting in improved productive and reproductive performance of dairy animals. The benefit of using herb is its affordability, ready accessibility and safety for health. Phyto-therapy is a traditional remedy for different diseases where plants mainly herbs and their products are used. Secondary metabolism in plants led to the production of an extensive array of organic compounds and owing to their chemical structure these compounds are beneficial nutritively for animals. In ruminant nutrition the so called phytonutrients or phyto-compounds which are derived from plants are bioactive in nature and have wide range of antimicrobial activities against several pathogens and proves to be efficient rumen modifiers (Oh et al. 2017). Their mechanism of action involves the binding to particular receptor present on a intestine, neuron and other cells which led to various physiological changes in non-ruminants *viz*.; immune responses, oxidative stress, and insulin secretion and activity. Certain phyto-compound follows the similar mode of action as in non-ruminant species. They are more resistant to microbial degradation in the rumen due to their phenolic makeup and may express post ruminal activities. However to the best of author's knowledge the information on beneficial effect of phyto-compounds on health and productivity during transition period in crossbred cows is negligible. Thus, it is the aim of present study to investigate the effects of phyto-pharmaceutical product on health and productivity in transition cows.

Materials and Methods

A total of twelve cross-bred cows (Karan-Fries; Holstein-Friesian × Tharparkar) in their advanced gestation were selected at 30 days pre-partum from the Livestock Research Centre, ICAR-National Dairy Research Institute, Karnal, Haryana. Institute Animal Ethic Committee (IAEC) duly permitted the experimental protocol. These cows were randomly allotted to SG and CON with six cows in each group on the basis of parity (2-5) and 305 day milk yield (4523.36 ± 248.84) kg. Both groups were fed as per NRC (2001). CON group was fed according to their nutritional requirements as per NRC standards while SG was supplemented additionally with 20g of poly-herbal formulation containing *Boswellia serrata* (Burseraceae) and 20g of *Berginia ciliata* (Saxifragaceae) for 21 days pre-partum to up to 21 days postpartum. The poly-herbal powder was prepared by CSIR-Indian Institute of Integrative Medicine, Jammu under the Department of Biotechnology approved research project entitled "*Development of Phyto-pharmaceutical product for Bovine Mastitis*". This product was tested in Jammu on immunosuppressant mice and then handed over to ICAR-NDRI, Karnal for testing on crossbred cows around the immunosuppressed period of parturition. To analyse the health status of cows blood samples were collected by puncture in jugular vein in the heparinised vacutainer tubes at -21, -14, -7, -3, 0, 3, 7, 14 and 21 days of parturition. TLC was estimated in fresh blood by haemocytometer. Mehrzad et al. (2004) method was followed to estimate the blood neutrophils in plasma samples. At -20°C plasma samples were stored for further study. Concentrations of viable neutrophils concentration were adjusted to 5.0x10^6 viable neutrophils/ml of PBS. Modified colorimetric nitro blue tetrazolium (NBT) reduction assay method was used to determine the PA of blood neutrophils (Chai et al. 2005). Blood plasma cortisol, IL-2 and IL-8 were estimated by bovine ELISA test kit (Endocrine Technologies, USA). The milk constituents such as fat, protein and lactose were analysed by Automatic Lactoscan milk analyser. Milk somatic cells were estimated by Lactoscan milk SCC counter (Milkotronic Ltd. Stara Zagora, Bulgaria).

Statistical analysis

Data were analysed statistically by two-way ANOVA with interaction to compare the effect of supplementation as well as week wise variation during transition period. Data are presented as Mean ± S.E. The analysis was considered significant at P values less than 0.05.

Results and Discussion

The present experiment was conducted to determine the effect of phyto-pharmaceutical product on health and production of dairy cows. Results of this study are presented in Table 1. The mean total leukocyte counts (TLC) value between SG and CON up to 14 days pre-partum did not differ significantly ($P<0.05$), but from 7 day pre-partum to day of parturition mean TLC value decreased significantly ($P<0.05$) in SG. At 7 day postpartum TLC decreased in SG, whereas, from 14 day postpartum and onward mean TLC value was significantly ($P<0.05$) lower in SG.

The mean TLC values at 21st day prepartum were about 7.6 to 7.9 m/mm^3, respectively in the blood of CON and SG group of cows. A significant ($P<0.05$) increase was observed in the neutrophils % on the day of parturition as compared to 7th day pre-partum in both the groups as shown in Table 1. On comparison between the two groups, neutrophils increased significantly ($P<0.05$) in the CON as compared to the SG. Neutrophils remained higher in the CON during the postpartum days, whereas it decreased in SG during the postpartum days. Blood lymphocyte percent ranged from 67.5% to 68.25% in both the CON and SG of cows, respectively (Table 1). Although, lymphocytes decreased significantly ($P<0.05$) at the time of parturition in both groups, it was lower ($P<0.05$) in the CON as compared to the SG. Percent (%) lymphocytes remained lower in the CON throughout the course of experimentation as compared to the SG cows. Comparison of N: L values has been presented in Table 1. N: L ratio was lower at 21st day pre-partum but increased ($P<0.05$) on the day of parturition in both the group of cows. Moreover, it was seen that the N: L ratio increased ($P<0.05$) in the CON on the day of parturition. Both the group of cows then showed a decline in N: L ratio postpartum. In Table 1, the result of PA has been presented. There was no significant ($P<0.05$) difference at 28 days pre-partum in both the groups. PA of neutrophils further decreased significantly around parturition ($P<0.05$) in both the groups. There was significantly ($P<0.05$) more PA at 14 day postpartum in SG as compared to CON. This trend of increased PA was continued till 28 days postpartum. In Table 1, it has been shown that IL-2 and IL-8 concentrations were significantly ($P<0.05$) higher in CON on the day of parturition and during postpartum days as compared to SG. The result pertaining to cortisol levels has been depicted in Table 1. Cortisol level was increased significantly ($P<0.05$) in both the groups as cows approached to parturition then decreased and reached to basal level at 7 weeks postpartum. On the day of parturition cortisol level was significantly ($P<0.05$) less in SG as compared to CON. Table 2 depicted the change in the percentage of milk fat which did not follow specific pattern and fluctuated during different days of postpartum period. It was numerically higher in the CON as compared to SG. However, it showed higher ($P<0.05$) values at day 14 and 42 postpartum in the CON as compared to the SG. In Table 2, milk protein % was significantly ($P<0.05$) higher in the CON at the first three weeks postpartum, it was lower around

Table 1: Effect of feeding phyto-pharmaceutical product during trasition period on the blood cell counts, phagocytic activity, plasma cortisol and cytokine levels of crossbred cows (Mean±S.E.)*

Parameters	Group	Days peri-partum						
		-21 day	-14 day	-7 day	0 day	7 day	14 day	21 day
TLC (m/mm^3)	Control	7.98^{a}±0.16	8.48^{ab}±0.44	8.80^{a}±0.37	9.56^{c*}±0.30	9.74^{a}±0.85	8.12^{a}±0.47	8.06^{a}±0.39
	Supplemented	7.60^{a}±0.36	8.00^{ab}±0.36	8.16^{ab}±0.35	8.45^{b}±0.28	7.91^{a}±0.42	7.71^{a}±1.29	7.70^{a}±1.12
Neutrophil (%)	Control	28.75^{a}±0.25	31.53^{b}±0.26	32.24^{b*}±0.44	39.97^{d*}±0.55	34.52^{c*}±0.86	32.21^{b*}±0.63	29.12^{a*}±0.28
	Supplemented	28.75^{a}±0.25	30.17^{c}±0.27	30.92^{c}±0.30	34.25^{d}±0.20	31.79^{c}±0.36	30.65^{c}±0.36	26.76^{a}±0.42
Lymphocyte (%)	Control	67.51^{c}±0.53	64.91^{bc}±0.65	62.73^{b*}±0.63	54.15^{a*}±0.69	60.15^{b*}±0.52	61.87^{b*}±0.64	66.83^{c*}±0.87
	Supplemented	68.25^{d}±0.49	66.14^{c}±0.50	64.86^{b}±0.48	59.77^{a}±0.62	63.12^{b}±0.36	64.53^{b}±0.45	69.25^{d}±0.35
N:L	Control	0.42^{a}±0.01	0.48^{a}±0.01	0.51^{b*}±0.01	0.73^{c*}±0.02	0.55^{b*}±0.02	0.52^{b*}±0.01	0.43^{a*}±0.01
	Supplemented	0.41^{a}±0.01	0.45^{b}±0.01	0.47^{b}±0.01	0.57^{c}±0.01	0.50^{b}±0.01	0.47^{b}±0.01	0.38^{a}±1.12
P.A. (OD at 450nm)	Control	0.24^{c}±0.01	0.23^{c}±0.02	0.19^{b}±0.01	0.17^{ab*}±0.01	0.16^{a*}±0.01	0.19^{b*}±0.03	0.17^{ab*}±0.01
	Supplemented	0.23^{c}±0.01	0.22^{c}±0.01	0.19^{b}±0.01	0.19^{ab}±0.01	0.17^{a}±0.01	0.25^{cd}±0.01	0.25^{d}±0.01
IL-2 (pg/ml)	Control	301.75^{a}±27.52	354.25^{b}±24.97	374^{b}±45.18	437.75^{c*}±10.17	389^{b}±11.27	352.50^{b*}±21.12	311.75^{a*}±11.92
	Supplemented	305.25^{a}±19.35	372^{bc}±36.82	394.5^{bc}±27.02	407^{c}±11.07	353.25^{b}±14.57	301.00^{a}±12.96	270.75^{a}±13.09
IL-8 (pg/ml)	Control	459.5^{a}±33.96	468^{a}±17.73	483.5^{a}±36.71	527.25^{b}±23.08	536^{b*}±8.25	472.75^{a}±16.70	454.75^{a}±11.95
	Supplemented	451^{a}±14.39	443^{a}±4.91	448.25^{a}±20.08	503.25^{b}±23.08	453.75^{a}±22.40	450.25^{a}±32.32	426.75^{a}±35.16
Cortisol (ng/ml)	Control	3.19^{ab}±0.21	3.37^{ab}±0.31	4.65^{c}±0.29	8.58^{e*}±0.35	6.18^{d*}±0.57	3.83^{b}±0.57	2.71^{a}±0.39
	Supplemented	3.04^{ab}±0.37	3.22^{b}±0.14	5.08^{d}±0.21	6.99^{e}±0.53	4.38^{c}±0.35	2.93^{ab}±0.41	2.46^{a}±0.23

^{abc}superscripts within a row are significantly different (p<0.05) among the days within the group; asterisk (*) within the column indicates the significant difference (p<0.05) within same day between the groups.

peak lactation as compared to the SG. The milk lactose was always higher in the SG as compared to CON and showed significant (P<0.05) difference in the postpartum period. In Table 3, the milk SCC was lower (P<0.05) in the SG as compared to the CON. Results of SCC indicated that mammary health status of animals in SG were better and significantly (P<0.05) lower as compared to CON.

An increase in TLC around parturition is mediated by pre-partum rise in cortisol levels (Hussain and Daniel, 1992). It substantiates the fact that stress in different species leads to increase in TLC (Kumari et al. 2018; Mc-Glone et al. 1993). Prior to late gestation high concentrations of cortisol have been reported that facilitate parturition, thereby producing immunosuppressive and anti-inflammatory properties in cows (Nagel et al. 2019; Mordak and Anthony, 2015; Dang et al. 2013). Positive effect of supplementation of mixture in lowering plasma cortisol values was also observed in 28 days postpartum. Phytochemicals have excellent (%) DPPH radical scavenging activity (Al-Rehaily et al. 2002; Mishra et al. 2005; Riddhi and Yogesh, 2012); which is helpful in reducing stress. The previous studies (Kimura et al. 1999) indicated that peak neutrophil numbers are observed at parturition, but level declines shortly after and reach basal conditions within 2 weeks. The results of this study followed similar patterns. Around parturition increased neutrophil count was physiologically associated with high peri-parturient blood concentrations of glucocorticoids (Lee and Kehrli, 1998). PA is reduced during parturition as cortisol binds to the receptors of the neutrophils (Burton et al. 2005). Intracellular rise of reactive oxidative species (ROS) occurs during phagocytosis process that mediates inflammation, however, adversely affects the cell and surrounding tissue (Sharma et al. 2014). Due to this intracellular rise of reactive oxidative species (ROS), PA is potentially reduced owing to its low antioxidant status. Most of the fat soluble antioxidant vitamins such as retinol, α-tochopherol and β-carotene concentration decreased at the time of parturition leading to depression in PA (Weiss, 1998). Cellular and humoral immune function is improved by these vitamins due to chain breaking lipid soluble tissue antioxidant properties (Halliwell, 1987). Chaterjee (1994) observed increased microbicidal activity of neutrophils and elevated antibody titre in both primary and secondary immunity assay at the dose rate of 20mg/kg body weight herbal preparation (Immu-21) containing *Ocimum sanctum*, *Emblica officinalis*, *Withania somnifera*, *Tinospora cordifolia* on immunological properties in rats. The present study reported low levels of cytokine expression in SG on day of parturition as compared to CON which indicates the immunomodulatory properties of poly-herbal product. Tian et al. (2015) reported that the cytokine production, the rise in the surface receptors for other molecules or the inhibition of their own effect by feedback inhibition results due to the up and down regulation of various genes and their transcription factors due to the cytokine interaction with its cell surface receptors. Cellular immune system acts through

Table 2: Effect of feeding phyto-pharmaceutical product during trasition period on the milk composition of crossbred cows (Mean±S.E.)*

Parameters	Group	Days of lactation								
		0 day	7 day	14 day	21 day	28 day	35 day	42 day	49 day	56 day
Fat (%)	Control	$4.40^{ab}\pm0.07$	$4.68^{ab}\pm0.47$	$4.43^{ab*}\pm0.15$	$4.46^{ab}\pm0.18$	$4.13^{a}\pm0.18$	$4.94^{b}\pm0.84$	$4.84^{b*}\pm0.47$	$4.52^{ab}\pm0.26$	$4.19^{a}\pm0.32$
	Supplemented	$4.44^{b}\pm0.23$	$4.36^{ab}\pm0.16$	$4.13^{a}\pm0.04$	$4.38^{ab}\pm0.19$	$4.14^{a}\pm0.06$	$4.17^{a}\pm0.07$	$4.18^{a}\pm0.05$	$4.33^{ab}\pm0.15$	$4.00^{a}\pm0.04$
Protein (%)	Control	$3.68^{b}\pm0.26$	$3.93^{d*}\pm0.14$	$3.80^{cd}\pm0.18$	$3.58^{bc}\pm0.26$	$3.47^{bc}\pm0.33$	$3.44^{b}\pm0.29$	$3.42^{b}\pm0.13$	$3.20^{a*}\pm0.04$	$3.27^{ab}\pm0.10$
	Supplemented	$3.53^{ab}\pm0.24$	$3.45^{ab*}\pm0.16$	$3.61^{b}\pm0.19$	$3.56^{ab}\pm0.17$	$3.51^{ab}\pm0.13$	$3.52^{ab}\pm0.13$	$3.68^{b}\pm0.10$	$3.48^{ab*}\pm0.10$	$3.43^{a}\pm0.08$
Lactose (%)	Control	4.51 ± 0.27	4.45 ± 0.32	$4.29^{*}\pm0.17$	4.33 ± 0.41	$4.20^{*}\pm0.22$	$4.12^{*}\pm0.14$	4.18 ± 0.04	4.42 ± 0.12	4.32 ± 0.12
	Supplemented	$4.75^{bc}\pm0.13$	$4.66^{b}\pm0.05$	$4.80^{c}\pm0.08$	$4.69^{b}\pm0.21$	$4.69^{b}\pm0.11$	$4.51^{b}\pm0.14$	$4.28^{a}\pm0.19$	$4.62^{b}\pm0.20$	$4.56^{b}\pm0.13$

^{abc}superscripts within a row are significantly different ($p<0.05$) among the days within the group; asterisk (*) within the column indicates the significant difference ($p<0.05$) within same day between the groups.

Table 3: Effect of feeding phyto-pharmaceutical product during trasition period on the somatic cell counts x 10^5/mlin colostrum and milk of crossbred cows (Mean±S.E.)*

Parameter	Group	Days of lactatio								
		0 day	7 day	14 day	21 day	28 day	35 day	42 day	49 day	56 day
Somatic cell l	Control	$5.07^{c*}\pm0.12$	$2.92^{b*}\pm0.13$	$2.45^{a}\pm0.32$	$2.38^{a}\pm0.28$	$2.35^{a*}\pm0.17$	$2.42^{a*}\pm0.2$	$2.45^{a*}\pm0.25$	$2.55^{a*}\pm0.18$	$2.65^{a*}\pm0.13$
counts x10^5/m	Supplemented	$4.55^{c}\pm0.14$	$2.29^{b}\pm0.2$	$2.25^{b}\pm0.13$	$2.20^{b}\pm0.15$	$1.98^{ab}\pm0.11$	$1.85^{a}\pm0.19$	$1.88^{ab}\pm0.22$	$2.04^{ab}\pm0.13$	$2.22^{b}\pm0.15$

^{abc}superscripts within a row are significantly different ($p<0.05$) among the days within the group; asterisk (*) within the column indicates the significant difference ($p<0.05$) within same day between the groups.

their inflammatory cytokines which are multi-potential mediators and exhibits various biological activities. Barak (1995) reported that very low or high concentrations of these cytokines may lead to unfavourable effects. The duration of the immune response creates a balance between the production of inflammatory and anti-inflammatory cytokines (Bhatt et al. 2014). Kholef and Khorshed (2006) studied milk composition analysis and showed that milk protein was higher ($P<0.05$) in animals fed experimental additives than the control. In other studies, milk fat, protein and lactose contents were not affected by poly-herbal supplementation ration fed lactating goats (Campanile et al. 2008; Erasmus et al. 2005). This study suggested the anti-inflammatory effects of the supplementation. The study also reported lower SCC in SG as compared to CON. Bhatt et al. (2014) studied that supplementation resulted in low bacterial count which may be indicative of the enhanced phagocytosis in the tissues due to the cytokines in SG. Hillerton (1999) studied that somatic cell was closely related with inflammation, udder health and milk quality as well as it reflects the herd health status. Also Chandra et al. (2016) reported that poly-herbal mixture and butyric acid supplementation during transition period has beneficial effect in improving udder health status in the supplemented group. This study indicated that inflammatory cytokine expression and function of neutrophil was regulated by butyrate especially in the presence of inflammatory stimuli.

Conclusion

Thus, feeding of extracts of *Berginia ciliate* and *Boswellia serrata* during the peri-partum period may reduce postpartum stress and supplements boost the activity of immune cells around the parturition period of high milk producing cows.

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