Effects of supplementation of polyherbal-potash alum mixture on immune status of crossbred cows during transition period


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ABSTRACT

The present study was conducted to investigate efficacy of supplementation of polyherbal-potash alum mixture on immune status of cows during transition period. Fourteen crossbred cows in their late gestation were selected and randomly divided into 2 groups. Cows in one group were supplemented with mixture containing herbs and potash alum from 30 days ante-partum to 30 days postpartum. Trachyspermum ammi Asparagus racemosus, Ocimum sanctum, Emblica officinalis, Cinnamomum zeylanicum and potash alum were used for preparation of mixture. Immune status viz, total leucocyte counts (TLC), differential leucocyte counts (DLC), in-vitro phagocytic activity (PA) of blood neutrophils and plasma cortisol level were estimated as well as incidence of diseases were studied. Results showed that supplementation mixture significantly (P<0.05) reduced cortisol level and TLC, whereas phagocytic activity was increased on the day of parturition. However, no significant (P<0.05) changes were observed in DLC. Incidence of mastitis, metritis and retention of foetal membrane (RFM) in control group was 28.57%, 28.57% and 14.28%, respectively whereas no such cases were found in treatment group. The study clearly indicated that supplementation of polyherbal-potash alum mixture enhance immunity during transition period.

Key words: Immune status, Phagocytic activity, Polyherbal-potash alum, Transition period.

INTRODUCTION

During transition period metabolism of cow shifts from demand of pregnancy to lactation. Due to this shift chain of physiological and biochemical reaction occurs like rise in production of reactive oxygen species (ROS) (Sordillo, 2005). Immune cells are most sensitive to ROS production, because their membranes contain high concentration of poly-unsaturated fatty acids which are very susceptible to peroxidation. This impairment cause immune system related disorders like metritis, mastitis and retention of foetal membrane (Block, 2010). Uses of antibiotic to cope up these diseases cause antibiotic resistance as well as antibiotic residue within the animal products.

Strengthening the non-specific immunity by reducing the stress is the only perspective to overcome the incidence of diseases during the transition period. To potentiate non-specific immunity, it is essential to reduce stressors and ROS production. Bioactive compounds (e.g. thymol, cinnamaldehyde, cineol, methyl eugenol, anethol, allicin etc.) present in herbs have been used as immunomodulator to prevent diseases, enhance performance in stress related syndromes and increase resistance against infection. These natural antioxidants can be used as side-effect free alternative. Many researches have been done to prove the in-vitro antioxidant potential of these herbs. Ocimum extract has excellent antioxidant potential due to its highest in-vitro 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger activity(Riddhi and Yogesh, 2012). Tacouri et al. (2013) in an in-vitro analysis reported that Trachyspermum and Cinnamon are rich in total flavonoids content which is responsible for their excellent antioxidant and antimicrobial properties. They have a wide range of biochemical activities, including inhibition of ROS generation, direct or indirect scavenging of free radicals as well as alteration of intra cellular redox potential (Abdollahi et al., 2005). Sharma et al. (2014) reported that 250 mg/Kg polyherbal mixture can successfully enhance immunity by reduction of stress during late gestation. Reports are also available regarding ante-partum as well as postpartum supplementation of Asparagus root powder @100mg/kg B.wt. and 200mg/kg B.wt., respectively for significant improvement of immunity by increase in total milk immunoglobulin (Kumar et al., 2014). Hashemzadeh-Cligar et al. (2014) also reported that supplementation of 185g phytobiotic rich herbal extract containing dried cinnamon bark (18%) have strong lipophilic and hydrophilic antioxidant properties. In Indian ethno veterinary supplementation of potash alum during transition period is
a usual practice. It has been reported that mixture of potash alum, Brassica compestris and seed oil of Curcuma long can be given to animals orally for treatment of internal wound (Nag et al., 2007). Keeping in view the aforesaid facts, the present study was undertaken to investigate an alternative approach towards potentiating the immune system of dairy cows with the use of natural ingredients.

MATERIALS AND METHODS

The present experiment was conducted at 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 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 experiment dried herbs viz. Trachyspermum ammi, Asparagus racemosus, Ocimum sanctum, Emblica officinalis, Cinnamomum zeylancium and potash alum were stored in cool and dry place. These were pulverised and mixed. Mixture was prepared by mixing each pulverised ingredients in 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 on the basis of 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 acted as treatment. At the start of experiment (30 days ante-partum) cows were weighed and they were subjected to polyherbal-potash alum mixture as per their average body weight. Feeding was done as top dressing on concentrate mixture in morning up to 30 days postpartum.

Table 1: Composition of Polyherb-Potash alum mixture.

<table>
<thead>
<tr>
<th>Herbs</th>
<th>Amount (mg/kg B.wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trachyspermum ammi</td>
<td>50</td>
</tr>
<tr>
<td>Asparagus racemosus</td>
<td>100</td>
</tr>
<tr>
<td>Ocimum sanctum</td>
<td>50</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>50</td>
</tr>
<tr>
<td>Cinnamomum zeylancium</td>
<td>50</td>
</tr>
<tr>
<td>Potash Alum</td>
<td>10</td>
</tr>
</tbody>
</table>

To assess the immune status of cows, blood samples were collected in 10 ml heparinised vacutainer (BD Vacutainer® UK) tubes at -28, -21, -14, -7, -3, 0, 3, 7, 14, 21 and 28 days of calving. TLC was estimated in fresh blood by haemocytometer. Slides were prepared for DLC and then stained with Leishman’s stain. Blood neutrophils were separated by the method described by Mehrzad et al. (2004). Plasma samples were stored at -20°C for further analysis. Live neutrophils concentration was adjusted to 5x 10⁶ cells /ml by counting with trypan blue dye. Phagocytic activity (PA) of blood neutrophils was measured as per modified colorimetric nitro blue tetrazolium (NBT) reduction assay method (Rainard, 1986). Blood plasma was used for evaluating cortisol level by ELISA kit (Endocrine technology). For incidence of diseases occurred postpartum cows were observed daily and their milk samples were examined on weekly basis. All analysis was done by using Sigma plot version 11 software. 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 ± SE. Level of statistical significance was set at P <0.05.

RESULTS AND DISCUSSION

Present study was done to estimate the influence of polyherbal-potash alum mixture on health and immune status of dairy cows. Results of this experiment are presented as follows. Level of Cortisol has been depicted in Figure (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 3 weeks postpartum. At the day of parturition cortisol level was significantly (P<0.05) less in treatment as compared to control group. Normally, a high rise in cortisol just prior to parturition is due to the rise in stress during parturition which occurs due to the activation of the feto maternal axis mainly due to rise in stress of parturition(Dang et al., 2013). Positive effect of supplementation of mixture in lowering plasma cortisol values was also observed in 28 days postpartum. Sharma et
al. (2014) also reported that supplementation of polyherbal mixture containing Asparagus (19%), Emblica (19%) and Ocimum (19%) increased ferric reducing ability of plasma (FRAPs) value in crossbred cows at the day of parturition. Haq et al. (2013) reported similar finding that supplementation of Emblica officinalis powder @ 200 mg Kg B.wt. for 60 days can significantly (P<0.05) reduce the stress in crossbred cows. These finding again supports the lesser stress as well as better antioxidiant status of cows in Treatment group.

The result pertaining to TLC values have been presented in Figure (2). The mean TLC value between treatment and control group up to 14 days ante-partum did not differ significantly (P<0.05), but from 7 day ante-partum to date of calving mean TLC value decreased significantly (P<0.05) in treatment group. At 3 day postpartum significant (P<0.05) change was not observed between treatment and control groups. At 7 day postpartum TLC decreased significantly (P<0.05) in treatment group. An increase in TLC around calving is mediated by ante-partum rise in cortisol levels (Hussain and Daniel, 1992). It substantiates the fact that stress in different species leads to increase in TLC (Mc-Glone et al., 1993). Phytochemicals (Trachyspermum-Thymol, p-cymene, terpinene, á-pinene, terpinene-4-ol,terpinene, p-cymene; Cinnamon-Cinnamaldehyde, eugenol, trans-cinnamic acid; Ocimum-Cineole, eugenol, ursolic acid, cirsilineol; Emblica-phenol, tannins, flavonoids, glycocides, proanthacyanides; Asparagus-saponin, flavonoids, polyphenol and vitamin C) have excellent (% DPPH radical scavenging activity (Riddhi and Yogesh, 2012; Al-Rehaily et al. 2002; Mishra et al. 2005); which is helpful in reducing stress.

Comparison of DLC values has been presented in Table 2. Neutrophil (%) was increased significantly (P<0.05) in both the groups as cows approached to parturition and then decreased away from parturition. Significant (P<0.05) change was not observed between the groups for Neutrophil (%). In consonance previous studies (Kimura et al., 1999) indicated that peak neutrophil numbers are observed at parturition, but level declines shortly after and reach basal

![Fig 2: Total leucocyte counts in the blood of treatment and control group of cows during peripartum.](image-url)
conditions within 2 weeks. Result of this study followed this pattern. Physiologically high periparturient blood concentrations of glucocorticoids have been suggested as an explanation for the increased neutrophil count around parturition (Lee and Kehrl, 1998). Leucocyte (%) was decreased significantly (P<0.05) around parturition in both the groups. Reduction in lymphocyte (%) was associated with increase in neutrophil (%). No significant (P<0.05) changes were observed in monocyte, eosinophils and basophils during this period. Sharma et al. (2014) also reported that supplementation of polyherbal mixture containing Asparagus, Ocimum, Withania, Emblica, Tribulus and Nigella @ 200-250 mg/Kg B.wt. has no significant (P<0.05) effect on DLC.

The result of PA has been presented in Figure (3). At 28 days ante-partum there was no significant (P<0.05) difference found in both the groups for phagocytic activity of neutrophils. As these cows approached to parturition phagocytic activity of neutrophils decreased significantly (P<0.05) in both the groups. At 7 day ante-partum PA was significantly (P<0.05) less in control group as compared to treatment group. This trend of increased PA was continued till 28 days postpartum. During parturition, the cortisol binds to the receptors of the neutrophils and reduces their phagocytic ability (Burton et al., 2005). The phagocytic process is accompanied by an intracellular increase in ROS

![Fig 3: Phagocytic activity in blood neutrophil of control and treatment group of cows](image)

that is necessary to kill the pathogen but potentially dangerous to the cell and surrounding tissue (Sharma et al. 2014). Accumulation of ROS in PMN cells due to poor antioxidant status is generally associated with reduced phagocytic activity. Depression in phagocytic activity might be due to decreased concentration of most of the fat soluble antioxidant vitamins such as retinol,a-tocopherol and β-carotene at the time of parturition (Weiss, 1998). These vitamins improve cellular and humoral immune function due to chain breaking lipid soluble tissue antioxidant properties (Halliwell, 1987). Chaterjee (1994) studied the effect of herbal preparation (Immu-21) containing Ocimum sanctum, Emblica officinalis, Withania somnifera, Tinospora cordifolia on immunological properties in rats and observed increased microbicidal activity of neutrophils and elevated antibody titre in both primary and secondary immunity assay at the dose rate of 20 mg/kg body weight. Sharma et al. (2014) also reported supplementation of polyherbal mixture containing Asparagus, Ocimum, Emblica, Tinospora, Withania and Nigella @200-250 mg/Kg B.Wt. significantly (P<0.05) increased PA in treatment group. Therefore, it can be inferred from the results that supplementation of this mixture improved phagocytic activity of PMN and its effect can be attribute to antioxidant property of its ingredients.

Immune status has its significance only when the incidence of diseases could be less. Result depict that supplementation has positive effect reducing incidence of disease. In this study incidence of mastitis, metritis and retention of foetal membrane (RFM) in control group was 28.57%, 28.57% and 14.28%, respectively whereas no such cases were found in treatment group.

**CONCLUSION**

The results of the present study depict that supplementation of polyherbal-potashalum to be an effective strategy to solve the problem of immunosuppression in cows especially during transition period. It enhances the immunity as well as aid in reducing stress. It can be applied as suitable alternative for improving nonspecific immune system of periparturient cows. Further experiments are required on dose response relationship; formulation and standardisation to substantiate more promising recommendation.

**REFERENCES**


