Alteration in hematological profile of pregnant crossbred cattle of Assam after feeding some medicinal leaves/herbs and polyherbal preparation (Restobal) during their transition period

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Abstract
The present investigation was undertaken to study the alteration in the haematological profile of pregnant crossbred cows after feeding them with some local herbs/leave and Restobal. Twelve multiparous pregnant crossbred cows (Jersey X Non descriptive local cows) of Assam were selected for the experiment which was divided into five groups. Group I was control without any treatment and Group II was fed Neem leaves (Azadirachta indica) the last three months of pregnancy to three months after parturition. Group III was fed with bogori leaves (Zizyphus mauritiana) orally. Group IV and group V were fed bamboo leaves and Restobal, a commercially available polyherbal preparation respectively daily from the last three months of pregnancy to three months after parturition. The RBC count ranged from (10^6/µl) 6.32 ± 0.04 to 7.10±0.06 in the control group, 6.44 ± 0.05 to 7.35 ± 0.07 in group II, 6.30±0.01 to 6.64 ± 0.15 in group III, 6.34 ± 0.05 to 7.17 ± 0.12 in group IV and 6.39 ± 0.05 to 6.68 ± 0.12 in group V. A significant increase in the level of RBC count in group II on the day of calving (P < 0.01) and 15 days prior to parturition (P < 0.001) and till 45 days after parturition as compared to the control group might be due to the effect of feeding the animals with Neem leaves. Group IV animals those were given Restobal also showed an increase in the overall mean RBC count than that of the control group. The level of hemoglobin ranged from 10.05 ± 0.145 to 11.23 ± 0.236 (gm/dl) in the group I (control); 10.08 ± 0.189 to 11.24 ± 0.244 in group II, 10.33± 0.167 to 11.67 ±0.134 in group III, 10.42 ± 0.31 to 11.15 ± 0.405 in group IV and 10.22 ± 0.26 to 11.25 ± 0.245 in Group V during the experimental period. All the groups showed an apparent rise in the level of haemoglobin than that of control group. Analysis of variance has shown that overall mean of WBC count in Group II and III were found to be higher than that of the control group but the increase was not significant. Group III animals that were fed with Bogori leaves also showed a non significant increase in the WBC count than the control group. From the present study it can be concluded that the locally available medicinal herbs/leaves and Restobal did not have any deleterious effect on the animals during their transition period and their supplementation might be helpful in the maintenance of pregnancy and lactation.

Keywords: Haematological, pregnant, lactation, herbs, multiparous

1. Introduction
The transition period, 3 weeks before parturition and 3 weeks after parturition is a crucial period for health, reproduction, production and profitability of cows (Bertoni et al.2008) [1]. During this period cows encounter drastic physiological changes and various stress factors like increased mobilization of body lipid, oxidative stress, altered endocrine status and immune function that results in increased risk of diseases (Jonsson et al. 2013) [14]. The transition period in dairy cattle, is characterized by a negative energy balance and micronutrient deficiencies (Drackley et al. 2001) [9]. Reproductive diseases during pregnancy and after parturition are a serious threat to the dairy cattle population often leading to abortion, still birth, delay in the recovery of ovarian function, involution of uterus at postpartum. The occurrence of the reproductive diseases lowers the production and reproduction of the animals leading to heavy loss to the dairy farm industry. The physiological changes occurring in the body of the dairy animals during this period have effect on the metabolic profile of cows around calving. During transition period a major change occur in blood cell parameters as dairy cows undergo a tremendous set of metabolic adaptations from late pregnancy to early lactation (Grummer, 1995) [10]. Scarcity of feed resources has be of the main limitation in the production of livestock products to meet the animal protein requirements of human as well as other industrial needs. Due to the stiff competition for feed stuffs’ energy and protein between humans and...
livestock, some easily available and cheaper novel feed resources have been focused areas of recent research (Mahmud et al. 2015) [15]. Neem (Azadirachta indica) commonly known as Nimba/holy tree belongs to the family Meliaceae is declared as the tree of the 21st century (United Nations Declaration). Neem leaf contains approximately 20.69% crude protein and 4.1% fat after processing into neem meal via drying and milling (Oforjindu, 2006) [17]. Ziziphus mauritiana (Bogori) belongs to family Rhamnaceae and commonly known as Indian jujube. The chemical compositions of the leaves are proteins & amino acids, flavonoids, alkaloids, glycosides, terpenoides, saponins, fibers, tannins and phenolic compounds. Restobal, a commercial herbal preparation been developed to improve the health of livestock and enhance productivity without compromising on safety and sustainability. Bamboo leaves make an essential component of ruminant rations and can provide green fodder almost throughout the year (Datt et al. 2006) [4]. Despite the remarkable contribution of research on physiology and nutrition of transition cows, the transition period still remains an area of concern for many dairy farms, and metabolic disorders continue to occur at the commercial dairy farms (Burhans et al. 2003) [13]. Nutrition management may be an essential means to improve the reproductive performance dairy cows (Ferguson and Chalupa, 1989) [9]. So far the consequences of feeding the locally available medicinal plant leaves/herbs on the hematological profile during transition period have not been studied in details in crossbred jersey cows (Jersey X Non descriptive local cows) of Assam. Considering these points, the present research was undertaken to unravel the effect of some locally available herbs/plant leaves on the hematological profile pregnant crossbred cows (Jersey X Non descriptive local cows) of Assam.

2. Materials and methods

Twelve numbers of multiparous pregnant crossbred cows (Jersey X Non descriptive local cows) of Assam were selected from the Instructional Livestock Farm (Cattle), College of Veterinary Science, AAU, Khanapara, Guwahati, Assam. Selected animals were divided into five groups keeping six in each group. Group I was control without any treatment and Group II was fed neem leaves (Azadirachta indica) at the rate of 3gm per kg body weight from the last three months of pregnancy to three months after parturition. Group III was fed with bogori leaves (Zizyphus mauritiana) at the rate of 300 mg per kg body weight orally. Group IV and group V were fed Restobal, a commercially available oral herbal preparation at the dose rate of 50 ml orally and bamboo leaves (Bambusa bambos) at the rate of 3gm per kg body weight daily from the last three months of pregnancy to three months after parturition. Blood samples were collected by puncturing jugular vein under aseptic conditions at different time period ie. On 90, 75, 60, 45, 30, 15 days prior to parturition and on ‘0’ day and three months after parturition at 15 days interval. The blood samples were collected between 9 am and noon in order to reduce the variation associated with the diurnal rhythms in blood. For the estimation of haematological parameters, blood samples with anticoagulant EDTA (ethylene-diaminetetraacetic acid) were collected. Total erythrocyte count (RBC) and the White blood count (WBC) were estimated by the routine method. The hemoglobin level was estimated by the Sahli’s acid haematin method and was expressed in gm %. The statistical analysis of the generated experimental data was done by the software, Graphpad prism (version 5.0).

3. Results and discussion

3.1 RBC count

Mean ± S.E of RBC count of the five different experimental groups of cows have been presented in Table 1. The RBC count ranged from (10^6/µl) 6.32 ± 0.04 to 7.10± 0.06 in the control group, 6.44 ± 0.05 to 7.35 ± 0.07 in group II, 6.30±0.01 to 6.64 ± 0.15 in group III, 6.34 ± 0.05 to 7.17 ± 0.12 in group IV and 6.39 ± 0.05 to 6.68 ± 0.12 in group V. On analysis of variance a significant increase in the level of RBC count was observed in group II on the day of calving (P < 0.01) and 15 days prior to parturition (P < 0.001) and till 45 days after parturition as compared to the control group. No significant difference was observed between group I and group IV. However, a non significant rise in RBC count was seen in group IV in the late gestation than that of the control group. All the groups showed a similar trend of increase in RBC count till late gestation and then a decline on the day of calving and thereafter which corroborates with the findings of Talvelkar et al. (2006) [29] who found that RBCs count was significantly higher on the day of calving, 1 week prepartum and fall significantly 1 week postpartum in buffaloes. Our findings are in agreement with Nazifi, et al. (2002) [10] also reported significantly higher RBC count in pregnant cows than that of postpartum cows in 55-60 days after parturition. Fetal growth that occurs in that period of pregnancy produces a greater oxygen demand which is compensated by the endocrine system that stimulates the release of erythropoietin by the renal tissue (Plaschka et al., 1997) [20]. Gavan, et al. (2010) [8] observed that the value of RBC count decreased after parturition and then increased again in early to mid lactation in Holstein cows. The reduction in the erythrocyte count in pregnant animals may be related to the physiological anemia occurring due to hemodilution which occurs as consequence of increase of plasma volume (Singh et al., 1991[25], Ozegbe, et al. 2001[18]). No significant differences in RBCs count were observed among early, mid, late lactation groups, dry (pregnant) and healthy control groups in lactating buffaloes (Hagawane, et al.2009) [11]. Similarly, Flores et al. (1990) [6] also did not find any significant differences in RBC count values during gestation and early lactation in cows. A significant increase in the level of RBC count in group II on the day of calving (P < 0.01) and 15 days prior to parturition (P < 0.001) and till 45 days after parturition as compared to the control group might be due to the effect of feeding the animals with Neem leaves as it has been found that the Neem leaves possess haematostimulatory properties (Haque et al,2006) [12]. Group IV animals those were given Restobal also showed an increase in the overall mean RBC count than that of the control group. Restobal is a herbal product that comprises of herbs namely Ocimum sanctum, Withania somnifera, Phyllanthus emblica and many more in fixed concentration. Sivajoth et al. (2018) [27] reported reduced haemoglobin in buffaloes after 5th day of vaccination but in buffaloes treated with Restobal showed the maintenance of normal levels. There is no variation in the packed cell volume of both the group of buffaloes. An increase in the total erythrocyte count was recorded in the Restobal supplemented group of buffaloes which had a role in the immunity development. Group IV and Group V, showed a reduced RBC count than the control, however, they were within the normal range.
3.2 Haemoglobin

Mean ± S.E of hemoglobin level of the five different groups of cows during late gestation and early pregnancy have been presented in Table 2. The level of hemoglobin ranged from 10.05 ± 1.45 to 11.23 ± 0.236 (gm/dl) in the group I (control). 10.08 ± 0.189 to 11.24 ± 0.244 in group II, 10.33± 0.167 to 11.67 ±0.134 in group III, 10.42 ± 0.31 to 11.15 ± 0.405 in group IV and 10.22 ± 0.26 to 11.25 ± 0.245 in Group V during the experimental period. Analysis of variance showed no significant difference between the groups. Overall mean showed that there was a higher level of haemoglobin in group II followed by group IV, group III and II. All the groups showed an apparent rise in the level of haemoglobin than that of control group. Our result goes in accordance with the findings of Steinhardt et al. (1994) [28] who reported a decrease in haemoglobin level with advancing lactation with high increase at parturient stage in dairy cattle. The decrease in the level of haemoglobin during pregnancy could be due to the dilution of blood which occurs as consequence of increase of plasma volumes (Singh et al. 2002) [29]. The higher levels of hemoglobin on the day of calving and immediately after calving denotes increased requirement of energy by the body tissue for accomplishment of all the physiological processes related to calving (Patel et al.2017) [19]. An increase in the level of haemoglobin in group II as compared to the control group and the other groups might be due to the effect of feeding the animals with Neem leaves as it has been found that the Neem leaves possess haematostimulatory properties (Haque et al.2006) [32]. The high level of Hb in birds supplemented with A.indica (neem) leaf meal might be due to hepato-stimulatory and hepatoprotective effects of leaf meal resulting in more synthesis of Hb in the bone marrow which is under the control of erythropoietic factors released by hepatic cells (Browman et al. 1976) [3].The increased blood parameters could be related to the reported constituents of the extract (flavonoids and quercetin) that have been shown to have hematopoietic properties (Raja et al., 2011) [21].The higher level of haemoglobin in group IV might be due to the haematopoietic property of Restobal given to the animals. Sivajoth et al. (2018) [25] reported reduced haemoglobin in buffaloes after 5th day of vaccination but in buffaloes treated with Restobal product showed the maintenance of normal levels.

3.3 WBC count

The Mean ± S.E values of WBC count of the five different groups of cows during late gestation and early pregnancy have been presented in Table 3. The WBC count ranged from (10^9 μl ) 11.72 ±0.01 to 13.11 ± 0.04 in the control group , 11.77 ±0.04 to 13.31 ±0.002 in group II, 11.75 ± 0.05 to 12.92 ±0.06 in group III, 11.67 ± 0.01 to 12.82 ± 0.03 in group IV and 11.87 ± 0.02 to 12.65 ± 0.08 in group V. Analysis of variance has shown that overall mean of WBC count in Group II and III were found to be higher than that of the control group but the increase was not significant. No significant difference was observed between the control and treated groups. The WBC count in the animals showed an increasing trend up to the day of calving, highest being recorded on the day of calving. However, there was a decline in the WBC count after parturition. The WBC count recorded on -30, -15, 0 day was significantly higher than that recorded 90 day prior to parturition. Significant difference in WBC count was seen in group V, 30 days after parturition as compared to that of the control. Leucocytosis, occurring during pregnancy is due to the physiologic stress induced by the pregnant state (Fleming et al. 1975) [7]. Suppression of leukocyte functions in dairy cows might be associated with negative energy balance around calving and in early lactation. Sometimes blood leukocyte numbers and their functions change considerably around parturition, resulting in suppression of the immune response from a few weeks before to a few weeks after calving. The higher level of WBC count in group II might be due to the effect of feeding neem as it has been reported to boost the body's macrophage response, which stimulates the body's lymphatic system and also boosts the body's production of WBC (Ray et al.1996) [24].Group III animals that were fed with Bogori leaves also showed a non significant increase in the WBC count than the control group. Sivajoth et al. (2018) [25] reported significant elevation in the total leukocyte count and lymphocyte count compared with the buffaloes in group I on 5th day of therapy. Safizadeh (2017) [24] investigated the effects of high doses of Jujube fruits and Saffron petals on hematological biomarkers in rats. No difference reported in hematological parameters of rats after treatments and the study suggested that administration of high doses of Jujube do not exert any toxic effect. Hematological parameters are known to be influenced by various factors such as age, breed, sex, seasonal variations, lactation, pregnancy etc. (Hewett, 1974[13]; Sastry, 1989[24]). Bambo may help mitigate the effects of gastrointestinal nematodes (GIN), such as the barberpole worm (Hae-monchus contortus), a major parasite of small ruminants in the southern USA. Bambo may help mitigate the effects of gastrointestinal nematodes (GIN), such as the barberpole worm (Hae-monchus contortus), a major parasite of small ruminants in the south.

| Days | GROUP 1 | GROUP 2 | GROUP 3 | GROUP 4 | GROUP 5 |
|------|---------|---------|---------|---------|---------|
| -90  | 6.32 ±0.04 | 6.44 ±0.05 | 6.30 ±0.01 | 6.34 ±0.03 | 6.39 ±0.05 |
| -75  | 6.56 ±0.01 | 6.59 ±0.10 | 6.38 ±0.05 | 6.56 ±0.10 | 6.53 ±0.03 |
| -60  | 6.72 ±0.10 | 6.74 ±0.07 | 6.40 ±0.07 | 6.71 ±0.15 | 6.46 ±0.06 |
| -45  | 6.87 ±0.11 | 6.90 ±0.05 | 6.46 ±0.22 | 6.89 ±0.03 | 6.53 ±0.03 |
| -30  | 7.06 ±0.13 | 7.19 ±0.14 | 6.49 ±0.12 | 7.06 ±0.11 | 6.57 ±0.01 |
| -15  | 7.10 ±0.06 | 7.35 ±0.07 | 6.54 ±0.10 | 7.13 ±0.16 | 6.67 ±0.10 |
| 0    | 7.05 ±0.09 | 7.27 ±0.11 | 6.64 ±0.15 | 7.17 ±0.12 | 6.68 ±0.12 |
| +15  | 6.88 ±0.12 | 7.04 ±0.12 | 6.60 ±0.11 | 6.94 ±0.22 | 6.62 ±0.15 |
| +30  | 6.65 ±0.05 | 6.89 ±0.18 | 6.55 ±0.15 | 6.75 ±0.25 | 6.53 ±0.06 |
| +45  | 6.58 ±0.11 | 6.79 ±0.07 | 6.50 ±0.06 | 6.67 ±0.11 | 6.51 ±0.08 |
| +60  | 6.60 ±0.02 | 6.73 ±0.06 | 6.53 ±0.02 | 6.63 ±0.02 | 6.53 ±0.05 |
| +75  | 6.62 ±0.03 | 6.76 ±0.08 | 6.55 ±0.01 | 6.66 ±0.15 | 6.55 ±0.01 |
| +90  | 6.63 ±0.01 | 6.80 ±0.16 | 6.64 ±0.03 | 6.67 ±0.19 | 6.61 ±0.15 |
| Overall | 6.74 ±0.06 | 6.89 ±0.074 | 6.51 ±0.028 | 6.79 ±0.064 | 6.56 ±0.023 |

Means bearing the same superscript in a row do not differ significantly in groups and means bearing same subscript in a column do not differ significantly in days.
Table 2: Haemoglobin (Mean ± S.E) (gm/dl) in Crossbred Jersey Cows after feeding with Medicinal Leaves/ Herbs And Restobal

| Days | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|------|---------|---------|---------|---------|---------|
| -90  | 10.73±0.156 | 10.62±0.102 | 10.60±0.082 | 10.75±0.056 | 10.72±0.179 |
| -75  | 10.72±0.200 | 10.60±0.183 | 10.82±0.065 | 10.97±0.098 | 10.70±0.180 |
| -60  | 10.83±0.214 | 10.77±0.220 | 11.07±0.115 | 10.95±0.307 | 10.82±0.222 |
| -45  | 10.85±0.198 | 10.88±0.227 | 11.37±0.130 | 11.20±0.142 | 10.88±0.252 |
| -30  | 11.10±0.266 | 11.08±0.232 | 11.57±0.134 | 11.15±0.405 | 11.08±0.231 |
| -15  | 11.23±0.236 | 11.24±0.244 | 11.67±0.133 | 10.88±0.595 | 11.25±0.245 |
| 0    | 11.07±0.219 | 11.03±0.208 | 11.52±0.183 | 11.73±0.555 | 11.03±0.207 |
| +15  | 10.68±0.212 | 10.75±0.198 | 10.60±0.068 | 10.68±0.523 | 10.73±0.206 |
| +30  | 10.55±0.180 | 10.58±0.166 | 10.33±0.17 | 10.67±0.507 | 10.63±0.199 |
| +45  | 10.37±0.174 | 10.42±0.194 | 10.33±0.167 | 10.58±0.46 | 10.48±0.224 |
| +60  | 10.35±0.167 | 10.32±0.168 | 10.63±0.18 | 10.52±0.39 | 10.47±0.228 |
| +75  | 10.25±0.188 | 10.25±0.186 | 10.72±0.21 | 10.42±0.31 | 10.37±0.221 |
| +90  | 10.05±0.145 | 10.08±0.189 | 10.73±0.16 | 10.47±0.22 | 10.22±0.26 |
| Overall | 9.15±0.085 | 9.64±0.117 | 9.43±0.10 | 9.59±0.119 | 9.25±0.110 |

Means bearing the same superscript in a row do not differ significantly in groups and means bearing same subscript in a column do not differ significantly in days

Table 3: WBC Count (10³/µl) (Mean ±S.E) In Crossbred Jersey Cows after feeding with Medicinal Leaves/ Herbs and Restobal

| Days | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|------|---------|---------|---------|---------|---------|
| -90  | 11.72±0.01 | 11.77±0.04 | 11.75±0.05 | 11.67±0.01 | 11.87±0.02 |
| -75  | 11.84±0.01 | 11.96±0.02 | 11.91±0.109 | 11.72±0.01 | 11.22±0.04 |
| -60  | 11.96±0.004 | 12.05±0.04 | 12.07±0.136 | 11.83±0.01 | 11.41±0.06 |
| -45  | 12.09±0.02 | 12.29±0.01 | 12.18±0.16 | 11.90±0.01 | 12.44±0.06 |
| -30  | 12.44±0.01 | 12.52±0.01 | 12.54±0.083 | 12.43±0.03 | 12.54±0.139 |
| -15  | 12.73±0.02 | 12.93±0.004 | 12.73±0.084 | 12.56±0.01 | 12.47±0.19 |
| 0    | 13.11±0.04 | 13.22±0.01 | 12.92±0.06 | 12.78±0.02 | 12.63±0.08 |
| +15  | 12.80±0.002 | 13.17±0.002 | 12.86±0.055 | 12.82±0.03 | 12.46±0.103 |
| +30  | 12.43±0.004 | 12.92±0.01 | 12.64±0.096 | 12.77±0.005 | 11.85±0.17 |
| +45  | 12.42±0.01 | 12.61±0.03 | 12.50±0.129 | 12.57±0.004 | 11.69±0.175 |
| +60  | 12.29±0.02 | 12.41±0.01 | 12.25±0.09 | 12.41±0.04 | 11.54±0.167 |
| +75  | 12.27±0.04 | 12.22±0.01 | 12.24±0.137 | 12.35±0.05 | 11.27±0.178 |
| +90  | 12.32±0.01 | 12.29±0.16 | 12.99±0.59 | 12.30±0.01 | 11.49±0.299 |
| Overall | 12.34±0.108 | 12.49±0.132 | 12.43±0.110 | 12.32±0.113 | 12.06±0.129 |

Means bearing the same superscript in a row do not differ significantly in groups and means bearing same subscript in a column do not differ significantly in days

4. Conclusion

Based on the results obtained from the present investigation it can be concluded that the locally available medicinal herbs/leaves did not have any deleterious effect on the animals during their transition period and the supplementation of these herbs and Restobal might be helpful in the maintenance of pregnancy and lactation. Variations in the hematological parameters between our findings and previous reports might be due to differences in sampling interval, methods used, numbers of cows sampled, degree of metabolic disturbances, genetic differences between cows, environmental conditions etc. However, more research on understanding the role of various medicinal plants/herbs on the hematological profile of cows will be helpful in improving health and optimizing production and profitability of dairy farms.

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