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Effect of feeding some medicinal leaves/herbs and herbal immunomodulator on serum total protein level in pregnant crossbred cows (Jersey X non descriptive local cows) of Assam

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Abstract
In the present study, 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 herbal immunomodulator respectively daily from the last three months of pregnancy to three months after parturition. The overall mean level of total protein (g/dl) was recorded as 8.38 ± 0.055 in group I, 8.40 ± 0.058 in group II, 8.37± 0.045 in group III, 8.39 ± 0.058 and 8.39 ± 0.054 in group IV and group V respectively during the experimental period. The group of animals fed with bamboo leaves showed total serum protein level of 8.39± 0.054 gm/dl which was non significantly higher than the control group. A significantly higher ($P<0.001$) level of protein was recorded 30 days after parturition than that of the day of calving. Overall mean level of total serum protein in Restobal fed group was found to be non significantly higher (8.39± 0.058 gm/dl) than the control group (8.38 ± 0.055 gm/dl).The present study revealed that the locally available medicinal herbs/leaves did not have any detrimental effect on the animals during their period of pregnancy and lactation and the supplementation of these herbs and the herbal immunomodulator might be helpful in the successful maintenance of pregnancy as well as lactation.

Keywords: Multiparous, pregnant, lactation, herbs, immunomodulator

Introduction

Pregnancy, parturition and onset of lactation impose a tremendous change to the homeostatic mechanisms of the cow leading to an increased risk of diseases including hypocalcemia, ketosis, hepatic lipidosis, laminitis, abomasal displacement, mastitis, retained placenta and metritis (Mulligan and Doherty, 2008) [13]. The period in pregnancy and around calving is also associated with high degree of inflammation (Bertoni *et al*., 2008) [3]. Dairy cows require essential amino acids, which are the building blocks of protein, for maintenance, growth, reproduction and milk production. These amino acids must come from either microbial protein synthesized in the rumen or from dietary protein that is not degraded in the rumen. The quantity of amino acids required by the lactating cow depends on the level of milk production. Plant and animal proteins are composed of more than 20 individual amino acids. Within the body, amino acids are used for a variety of structural proteins and enzymes; and they serve as a source of energy, carbon, and nitrogen. Amino acids and nitrogen are available to mammals through degradation of proteins and other nitrogenous compounds. Generally, the following amino acids are considered to be essential amino acids, because they cannot be synthesized by mammals: histidine, isoleucine, leucine, lysine, methionine + cystine, phenylalanine + tyrosine, threonine, tryptophan, and valine (NRC, 1989) [14]. Thus, these must be provided in adequate amounts by the diet. Other amino acids, such as arginine and taurine, may appear to be essential functionally during fetal and infant development in some species (Visek, 1986) [23]. The need of energy and protein during lactation increases dramatically. In dairy cows there is more than a 5-fold increase in energy and protein requirement from late gestation to lactation (Schingoethe *et al*) [21]. Protein malnutrition reduces the concentrations of most amino acids in plasma (Li *et al.*, 2007) [10]. 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). *Ziziphus mauritiana* 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. Leaves are used in the treatment of diarrhoea, fever, liver damage and pulmonary disorders (Gupta *et al*., 2012). [7] Restobal, a herbal immunomodulator 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) [5]. The effect of the locally available plant leaves and herbal immunomodulators during transition period have not been studied in details so far in crossbred jersey cows (Jersey X Non descriptive local cows) of Assam. Considering all these points, the present study was undertaken to assess the effect of some locally available herbs/plant leaves on the serum protein level in pregnant crossbred cows (Jersey X Non descriptive local cows) of Assam.

Materials and Methods

Twelve 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 for the experiment. Selected animals were divided into five groups keeping six in each. 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 herbal immunomodulator 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. General health of the animals, growth performance and weight gain by the calves were recorded daily for both the groups. 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. Serum separated from the blood was kept at-20 °C until used. Serum Constituents viz. total serum protein was estimated by Biuret method as described by Plummer (1971) [18] and was expressed in gm/dl. The statistical analysis of the generated experimental data was done by the software, *Graphpad prism* **(**version 5.0).

Result and Discussion

The mean serum profile of total protein late pregnancy and early lactation in five different groups of pregnant crossbred cows (Jersey X Non descriptive local cows) is presented in Table 1. The overall mean level of total protein (g/dl) was recorded as 8.38 ± 0.055 in group I, 8.40 ± 0.058 in group II, 8.37± 0.045 in group III, 8.39 ± 0.058 and 8.39 ± 0.054 in group IV and group V respectively during the experimental period. On analysis of variance it was found that there was significant difference in the level of serum protein between -90 day and 0 day i.e. on the day of parturition in all the groups. Higher level of serum protein was observed before parturition which declined on the day of calving and then subsequently showed an increase in its level after calving. A higher level of protein was found in group II and group V as compared to the control group which was not significant. A significantly higher (P<0.001) level of protein was recorded 30 days after parturition than that of the day of calving. This is similar to the findings of Piccione *et al.* (2012) [17] who observed that the total serum proteins levels were significantly affected from the physiological period and increased during lactation. The variations reflect the maternal requirements of proteins need for milking and providing immunoglobulin (Mohri *et al.*, 2007) [12]. Contrary to our finding serum total protein was found to be steadily decreased with increase in dietary Neem leaf meal although the differences were not statistically significant (P>0.05) (Obikaonu *et al*., 2012) [16].

Consistently high level of total protein has been seen by Yadav *et al.*, (2006) [24] in cyclic buffaloes as also observed in a study reinforcing the fact that high levels of plasma protein in the late trimester of pregnancy are needed for the optimum secretion of gonadotropin release factors and number of other hormones needed in the culmination of the pregnancy. Consistent with our finding, increasing pattern of serum total protein was also found in non-lactating Yaks by Pouroucholtamane *et al*., (2005) [19] and this phenomenon could be attributed to the non-lactating state of the cows in late trimester of the pregnancy.

The non significant rise in the level of protein group II might be due to the effect of neem feeding as it has been found that Neem leaves have higher crude protein concentration than most non-leguminous tree leaves. This coupled with a low level of fibre make it suitable as a protein supplement for ruminants on poor quality diets (Adjorlolo *et al*., 2016) [1]. On the other hand the significant depression in the total protein concentration might be ascribed to the fact that the fetus synthesis all its proteins from the amino acids derived from the mother, and maximum levels, especially in muscles, during late pregnancy (Yokus and Cakir, 2006) [25]. The high levels of total protein in the late trimester of pregnancy confirm the fact that high level of protein are needed for optimum secretion of ganadotropin release factors and number of other hormones needed in the culmination of the pregnancy (Mir *et al.*, 2008) [11]. In an experiment carried out by Obikaonu *et al.*, (2011) [15] serum protein elevated in birds fed with neem leaf meal at 1.252.5% levels than controls. Serum protein actually depends on availability of dietary protein. This means that the proteins of the leaf meal diets were more available to the birds confirming the recently observation by. The results are in accordance with the findings of Samarth *et al*., (2003) [20] who reported that herbs (Neem) increased the serum proteins as compared to control.

Ziziphus mauritiana (Bogori Leaves) possesses anti-inflammatory, cytoprotective, anti-allergic and antiulcer activity. The leaves also possess immunostimulant and cardiovascular properties. *Ziziphus mauritiana* leaves contain 13-17% crude protein and 15% fibre, and make an excellent fodder for livestock. In winter, the shoots and fruit of the trees are an important feed source (Hocking, 1993) [9]. Many other biological and pharmacological properties of *Ziziphus mauritiana* are yet to come. The Bogori (*Ziziphus mauritiana)* leaves fed group showed a non significant reduction of serum protein level than the control group.

A clinical trial was conducted by Sena *et al.*, 2012 [22] to study the effect of herbal immunomodulator during summer stress among camel calves. The results obtained indicated a significant change ($P<0.05$) in immunoglobulins, serum protein and globulin of Restobal fed group in comparison to control group and was concluded that supplementing herbal immune modulator Restobal augmented the growth rate, prevented the summer stress effect and maintained healthy immune system in camel calves of 5–6 months age. This is in line with our findings where overall mean level of total serum protein in Restobal fed group was found to be non significantly higher (8.39± 0.058 gm/dl) than the control group (8.38 ± 0.055 gm/dl)

Research has been conducted on the use of bamboo leaves as fodder for several animal species, including cattle, sheep and goats (Halvorson *et al*., 2010) [8], in many Asian and African countries. The use of bamboo leaves as fodder in several developing countries needs to be further developed and tested (Antwi-Boasiko *et al*.,2011) [2] Bamboo provided an important constituent of daily rations for the ruminants (Bhandari *et al*., 2015) [4]. The high protein and mineral contents of bamboo leaves complemented the high energy content and intake of maize silage (Delaby & Peyraud (2009) [6].The group of animals fed with bamboo leaves showed total serum protein level of 8.39± 0.054 gm/dl which was non significantly higher than the control group. A deficiency of dietary protein or amino acids has long been known to impair immune function and increase the susceptibility of animals to infectious disease.

The present study revealed that the locally available medicinal herbs/leaves did not have any detrimental effect on the animals during their period of pregnancy and lactation and the supplementation of these herbs and the herbal immunomodulator might be helpful in the successful maintenance of pregnancy as well as lactation. However, more knowledge about the role of various medicinal plants/herbs is necessary for the development of effective means to improve health, prevent and treat various diseases in animals.

Table 1: Total Protein (G/Dl) (Mean ± S.E) In Crossbred Jersey Cows after Feeding With Cytokine Stimulator/Regulator

Days	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 5
-90	$8.38^{a}_{A} \pm 0.04$	$8.31^{ab}_{A} \pm 0.04$	$8.36^{abc}_{A} \pm 0.04$	$8.31^{abcd}_{A} \pm 0.01$	$8.34^{abcd}_{A} \pm 0.01$
-75	$8.36^{a}_{AB} \pm 0.03$	$8.32^{ab}_{AB} \pm 0.05$	$8.42^{abc}_{AB} \pm 0.01$	$8.32^{abcd}_{AB} \pm 0.08$	$8.36^{abcd}_{AB} \pm 0.01$
-60	$8.37^{a}_{ABC} \pm 0.02$	$8.32^{ab}_{ABC} \pm 0.03$	$8.42^{ac}_{ABC} \pm 0.01$	$8.36^{abcd}_{ABC} \pm 0.02$	$8.37^{abcd}_{ABC} \pm 0.05$
-45	$8.39^{a}_{ABCD} \pm 0.01$	$8.33^{ab}_{ABCD} \pm 0.01$	$8.44^{ac}_{ABCD} \pm 0.01$	$8.37^{abcd}_{ABCD} \pm 0.02$	$8.43^{acd}_{ABCD} \pm 0.01$
-30	$8.28^{a}_{ABCDE} \pm 0.01$	$8.32^{ab}_{ABCE} \pm 0.01$	$8.33^{abc}_{ABCE} \pm 0.05$	$8.31^{abcd}_{ABCDE} \pm 0.07$	$8.28^{abcd}_{ABCE} \pm 0.01$
-15	$8.23^{a}_{FE} \pm 0.01$	$8.26^{ab}_{ABCDEF} \pm 0.01$	$8.26^{abc}_{AEF} \pm 0.00$	$8.27^{abcd}_{ABCDEF} \pm 0.04$	$8.25^{abcd}_{ABEF} \pm 0.04$
0	$7.99^{a}_{G} \pm 0.03$	$8.08^{ab}_{G} \pm 0.04$	$8.04^{abc}_{G} \pm 0.04$	$8.02^{abcd}_{G} \pm 0.01$	$8.00^{abcd}_{G} \pm 0.02$
+15	$8.09^{a}_{HG} \pm 0.03$	$8.13^{ab}_{HG} \pm 0.02$	$8.06^{abc}_{HG} \pm 0.03$	$7.98^{dc}_{HG} \pm 0.04$	$8.13^{abc}_{H} \pm 0.06$
+30	$8.43^{a}_{ABCDI} \pm 0.04$	$8.41^{ab}_{ABCDEI} \pm 0.03$	$8.39^{abc}_{ABCDEI} \pm 0.04$	$8.47^{abcd}_{ICD} \pm 0.03$	$8.45^{abcd}_{ABCDI} \pm 0.03$
+45	$8.55^{a}_{J} \pm 0.02$	$8.57^{ab}_{J} \pm 0.002$	$8.51^{abc}_{JBCD} \pm 0.06$	$8.58^{abcd}_{JI} \pm 0.04$	$8.62^{abd}_{J} \pm 0.02$
+60	$8.62^{a}_{KJ} \pm 0.04$	$8.69^{ab}_{K} \pm 0.00$	$8.52^{ac}_{KBCDJ} \pm 0.06$	$8.59^{abcd}_{KJ} \pm 0.02$	$8.61^{abcd}_{KJ} \pm 0.01$
+75	$8.63^{a}_{LJK} \pm 0.01$	$8.70^{ab}_{LK} \pm 0.03$	$8.53^{ac}_{LCDJK} \pm 0.04$	$8.61^{abd}_{LJK} \pm 0.05$	$8.62^{abcd}_{LJK} \pm 0.01$
+90	$8.63^{a}_{JKL} \pm 0.02$	$8.72^{ab}_{KL} \pm 0.04$	$8.54^{ac}_{DJKL} \pm 0.04$	$8.67^{abcd}_{LJKL} \pm 0.004$	$8.60^{acd}_{JKL} \pm 0.04$
Overall	8.38 ± 0.055	8.40±0.058	8.37± 0.045	8.39± 0.058	8.39± 0.054

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: Analysis Of Variance for Total Protein

Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	48	0.5799	0.01208	4.891****
group	4	0.04295	0.01074	4.347****
days	12	13.25	1.104	447.0**
Residual	325	0.8028	0.002470	

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