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RESEARCH ARTICLE

Effect of plane of nutrition on haematological and blood biochemical profile of pregnant Murrah buffaloes

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Abstract: This research assessed the impact of enhanced metabolizable energy (ME) and metabolizable protein (MP) supplementation in the final trimester of pregnancy on hematological and biochemical parameters in Murrah buffaloes. Forty pregnant buffaloes were allocated into four groups, each receiving diets with varying levels of metabolizable energy (ME) and metabolizable protein (MP): control (100% ME and MP), HME (130% ME, 100% MP), HMP (100% ME, 140% MP), and HMEMP (130% ME, 140% MP). Monthly blood samples collected until parturition were analyzed for haematological indices, plasma glucose, total protein, albumin, blood urea nitrogen (BUN), creatinine, immunoglobulins, liver enzymes, and antioxidant status. The findings indicated that buffaloes receiving higher protein and energy diets (HMP and HMEMP) exhibited significantly enhanced red blood cell counts, hemoglobin concentration, packed cell volume, plasma glucose, total protein levels, BUN, immunoglobulin concentration, Cortisol and SOD antioxidant activity in comparison to the control group. Increased BUN levels in these groups indicate heightened protein metabolism. No notable alterations were detected in leukocyte profiles. The findings indicate that increased dietary ME and MP in late pregnancy positively affect maternal physiological and overall health status, potentially enhancing fetal development and the postnatal health of calves. This nutritional strategy of enhancing metabolizable protein and energy may enhance health, welfare and reduce stress in buffaloes by altering at hormonal level.
Keywords: Biochemical, Buffalo, Energy, Haematological, Protein, Pregnancy

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Introduction

India's agricultural system primarily consists of a mixed crop-livestock farming model, wherein livestock significantly supports farmers. Livestock contribute to employment, serve as draught animals, and supply manure, thereby playing a crucial role in the livelihoods of rural India. India leads globally in buffalo population, housing approximately 108.7 million buffaloes, representing 56.53% of the worldwide buffalo population and 97.06% of that in Asia (FAOSTAT, 2015). Buffaloes are favored over cattle in tropical climates because of their enhanced capacity to utilize nutrients from low-quality fibrous tropical feeds and their superior adaptability to such environments (Chanda et al. 2021; Chiariotti et al. 2025). Malnutrition continues to be a major limitation on ruminant production in developing nations (Olafadehan and Adewumi, 2009). Non-productive animals, particularly those prior to calving, frequently experience neglect due to the high costs incurred by farmers without immediate returns (Bhatti et al. 2007). Besides nutrition, factors like healthcare and housing play a significant role in the growth rate of calves. Study by Radunz et al. (2012) show that the amount and quality of food given to domestic animals affect their growth after birth, how their bodies are made up, and how well they respond to insulin, which in turn impacts how efficiently they can produce in the future. Nutritional stressors during pregnancy, including inadequate nutrient supply from poor maternal nutrition or external environmental factors, can impede fetal development, subsequently influencing the growth and health of calves. The elevated rate of fetal growth imposes a greater nutritional demand on the dam, necessitating specific care to fulfill the metabolic requirements of both the fetus and the neonate. Calves are born with weak immune systems because their placental structure, called syndesmochorial, prevents the transfer of their mother's antibodies. Colostrum is crucial for delivering passive immunity to neonates and has been associated with maternal nutrition (Joshi et al. 1992). Calves born to undernourished dams during gestation exhibit increased neonatal mortality rates and heightened susceptibility to diseases (Odde, 1988). Blood urea nitrogen (BUN) is a critical parameter in the nutritional assessment of ruminants, indicating their protein and energy status. BUN levels rise with increased dietary protein intake, provided energy levels are stable. A deficiency in dietary protein results in reduced ammonia concentration in the rumen, subsequently decreasing BUN levels (Chase et al. 1993). Tatsapong et al. 2010 found that higher amounts of dietary protein lead to higher BUN levels, ranging from 17.0 to 20.7 mg% in animals with different protein diets. The association between dietary protein and blood urea nitrogen (BUN) concentrations is affected by the protein and energy content (Tatsapong et al. 2010). Kumar et al. (2013) examined the effects of dietary protein on haematological parameters and blood metabolites in growing Sahiwal calves. A protein level of 13.5% was determined to be adequate for sustaining normal blood parameters, such as BUN, total plasma proteins, immunoglobulins, and albumin. Shukla et al. (1994) showed that different protein sources influence plasma protein levels, with calves getting 100% energy levels having an average of 6.88 ± 0.24 g/dl, while those on 75% energy levels had an average of 6.50 ± 0.33 g/dl. Yazdani (2005) found that calves exhibited elevated plasma protein levels when provided with protein from cottonseed meal (CSM) in contrast to soybean meal (SBM). Varma et al. (2012) reported that plasma protein concentrations in growing female buffalo calves varied between 7.02 and 7.8 g/dl, indicating a moderate dietary impact on plasma protein levels. Immunoglobulin (Ig) levels, critical for evaluating immunity in ruminants, fluctuate with dietary protein consumption. Gupta et al. (2014) documented immunoglobulin levels of 25.40 mg/ml in Hariana heifers. Insulin-like growth factor 1 (IGF-1), an essential regulator of growth and development in ruminants, is notably affected by nutritional factors. Research conducted by Lee et al. (2006) demonstrated that the nutritional status of animals impacts serum IGF-1 levels, subsequently affecting growth and feed efficiency. A decrease in energy intake elevates growth hormone (GH) levels while reducing IGF-1 concentrations. The growth and health of ruminants, especially calves, are affected by multiple factors, including maternal nutrition, energy and protein intake, and environmental conditions such as season and stress. Maintaining balanced nutrition in the later stages of pregnancy is essential for optimal fetal development and the long-term health of the child. Also, understanding how immunoglobulins and growth factors like IGF-1 affect development after birth can improve how we manage livestock.

Based on the review, it was hypothesized that additional metabolizable energy and metablolizable protein or both in the dairy ration improves the performance of pregnant buffaloes. Therefore, this study was undertaken to determine the effects of feeding higher metabolizable energy (ME) and metabolizable protein (MP) in pregnant Murrah buffaloes on haemato-biochemical profile of pregnant Murrah buffaloes.

Material and Methods

The present study was conducted in three phases to study the effect of feeding higher metabolizable energy (ME) and metabolizable protein (MP) in pregnant Murrah buffaloes on hematological and blood biochemical parameters of pregnant Murrah buffaloes. The present experiment was conducted at Livestock Research Centre of ICAR-National Dairy research Institute, Karnal. Experimental protocol including handling and management of animals were in compliance with the prior approval of Institutional Animal Ethics Committee. The study was conducted over a duration of four months to evaluate the desired parameters. 40 Murrah buffaloes in last trimester (6 months) of pregnancy were selected and randomly divided into 4 groups (ten animals in each group) based on the body weight, lactation number and previous milk yield as control, high metabolizable energy (HME), high metabolizable protein (HMP) and high metabolizable energy and metabolizable protein (HMEMP) respectively to minimize the variation between groups. Control group was fed with 100% ME and 100% MP as per ICAR (2013) feeding standards, HME group were fed 100% MP and 130%ME, HMP group were fed 140% MP and 100% ME, and HMEMP group were fed with 130% ME and 140% MP of the ICAR (2013) feeding standard. Based on the ME and MP value four concentrate mixture was prepared, four different freshly prepared total mixed ration (TMR) was offered twice a day to control, HME, HMP and HMEMP groups, respectively. Fresh water was provided *ad libitum* four times daily. After proper restraining blood samples were collected from the jugular vein on monthly interval for hematological and blood biochemical analysis.

Ration formulation and feeding of animals

Four concentrate mixtures were formulated based on the ME and MP content of different ingredients. Mineral mixture and salt were added to the concentrate mixture at 2 and 1% of DM, respectively. The concentrate mixture was mixed with maize fodder and wheat straw in varying ratio to formulate four TMRs with different ME, MP and ME+MP. The animals were fed ration daily at 9.00 hour in the morning and 16.00 hour in the afternoon. After initial adaptation period of 7 days animals were tethered individually with rope on pucca floor having facility of individual feeding. Healthy surroundings and proper sanitary conditions were maintained throughout the experiment by regular spraying of phenyl on the floor and regular cleaning of mangers and walls of the shed.

Collection of blood

Blood was collected in the morning, before offering feed and water, at zero day and subsequently at monthly intervals. About 10 mL of blood was drawn from each animal in a sterile heparinised vacutainer (BD Vacutainer TM, UK) by jugular venipuncture, posing minimum disturbance to the animal. Just after the collection, tubes were rotated between palms ensuring proper mixing of blood with heparin. Immediately after collection, samples were placed in an ice box and transported to the laboratory and fresh blood was used for analysis of blood haematological parameters (Red Blood Cells (RBC), Hemoglobin

(Hb), packed cell volume (PCV), Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC)) by using BC-2800 Vet blood analyzer. Blood samples were centrifuged at 3000 rpm for 20 min to separate plasma, which was stored at –20°C in 2 mL Eppendorf vials awaiting further analyses of various plasma metabolites.

Plasma biochemical parameters were estimated using standardized commercial kits and established methods. Plasma glucose levels were measured using the Recombigen kit (India). Total plasma protein concentration was determined by the biuret method employing a commercial kit supplied by Recombigen Laboratories Pvt. Ltd., New Delhi. Plasma albumin was quantified via the bromocresol green (BCG) binding method. Blood urea nitrogen (BUN) and plasma creatinine levels were estimated using kits from Recombigen Ltd., with creatinine assessed by the modified Jaffe's method. Total immunoglobulins (Ig) in plasma were measured by the zinc sulphate turbidity method (Mc Ewan et al. 1970). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined kinetically using kits from Recombigen Pvt. Ltd. Hormonal analysis of cortisol was performed using a Bovine cortisol Enzyme-Linked Immunosorbent Assay (ELISA) sandwich kit (Bioassay Technology Laboratory, China). Antioxidant status was evaluated by estimating superoxide dismutase (SOD) activity in red blood cell hemolysates prepared within three days post-blood collection. All assays were performed following the manufacturers' protocols and standard laboratory procedures.

Results and Discussion

Haematological parameters

Effect of high energy and high protein diets on haematological parameters of pregnant Murrah buffaloes has been presented in Table 1.

The plane of nutrition effects the RBC count (x10^6/µl), Hemoglobin (g/dl) and PCV (%) of pregnant Murrah buffaloes significantly ($p<0.05$). It was observed that RBC count, Hemoglobin and PCV concentration was significantly high in HMEMP group and HMP group, intermediate in HME group and was least in Control group; it decreased with advancing pregnancy. At the starting of experiment RBC count in control, HME, HMP and HMEMP group was 8.53, 8.66, 8.68 and 8.46, respectively which was reduced to 5.70, 6.01, 6.37 and 6.33 before parturition in respective groups. The average hemoglobin (g/dl) content in control, HME, HMP and HMEMP group was 12.16, 12.39, 12.64 and 12.73, respectively. The mean PCV in control, HME, HMP and HMEMP group was 32.00, 32.26, 33.51 and 33.61. The levels of PCV were significantly high in buffaloes fed on high plane of nutrition as compared to control animals.

The TLC ranged from 9.83 to 11.01 in control group, from 9.72 to 10.66 in HME, from 9.83 to 10.70 in HMP and from 9.66 to 10.47 in HMEMP group. The total leucocyte count was statistically similar between different groups. The results showed non-significant ($p<0.05$) difference in DLC (%) i.e. lymphocytes, neutrophils, monocyte, eosinophils and basophil (%) values in control, HME, HMP and HMEMP group.

Blood biochemical parameters

Effect of high energy and high protein diets on Blood biochemical parameters of pregnant Murrah buffaloes has been presented in Table 2.

The plasma glucose (mg/dl) and total protein concentration of pregnant Murrah buffaloes was significantly ($p<0.05$) affected by dietary energy and protein levels.

The Plasma glucose concentration (mg/dl) values ranged from 49.59 to 68.08 mg/dl in control, 53.39 to 68.69 mg/dl in HME, 50.21 to 67.10 in HMP and 52.04 to 68.32 mg/dl in HMEMP groups, respectively. Overall, the levels of glucose was significantly ($p<0.05$) high in HMEMP group and HME as compared to HMP and control group.

Table 1: Effect of plane of nutrition on hematological parameters of pregnant Murrah buffaloes

	Control	HME	HMP	HMEMP
RBC (×10^6cell/µl)	6.96^b±0.17	7.16^b±0.16	7.85^a±0.17	7.92^a±0.17
Hemoglobin (g/dL)	12.16^b±0.14	12.39^{ab}±0.14	12.64^a±0.15	12.73^a±0.14
PCV (%)	32.00^b±0.45	32.26^b±0.40	33.51^a±0.40	33.61^a±0.50
TLC (×10^3cell/µl)	10.33±0.15	10.03±0.11	10.10±0.10	9.97±0.12
DLC (%)				
Lymphocytes (%)	57.12±1.14	58.90±1.16	59.42±1.07	60.09±0.91
Neutrophils (%)	33.08±1.08	31.01±1.11	30.31±1.12	29.70±0.94
Monocytes (%)	5.29±0.08	5.42±0.08	5.44±0.09	5.59±0.14
Eosinophils (%)	4.18±0.08	4.38±0.10	4.52±0.09	4.33±0.09
Basophils (%)	0.29±0.06	0.28±0.05	0.29±0.04	0.28±0.05

^{a,b,c,d} Means bearing different superscripts in a row differ significantly (P<0.05)

The plasma total protein concentration was significantly ($p<0.05$) affected by dietary energy and protein levels. The total protein concentration varied from 7.31 to 8.22, 7.21 to 8.48, 7.41 to 8.71 and 7.69 to 8.82 in control, HME, HMP and HMEMP, respectively. Critical analysis of data showed that plasma protein concentration was significantly ($p<0.05$) higher in HMEMP and HMP group, followed by HME and control group. Albumin levels (g/dL) in pregnant Murrah buffaloes fed different plane of nutrition ranged from 2.89 to 3.50, 2.94 to 3.40, 3.03 to 3.51 and 3.08 to 3.62 g/dL in control, HME, HMP and HMEMP groups, respectively. The albumin levels were statistically similar between HMP and HMEMP group and these levels were significantly ($p<0.05$) higher than control and HME group.

The biochemical parameter, plasma blood urea nitrogen (BUN), was found to vary with the plane of nutrition in pregnant Murrah buffaloes. The concentration of BUN was significantly ($p<0.05$) high in animals fed with 40% extra protein (HMP). The average mean of creatinine (mg/dL) in control, HME, HMP and HMEMP group was 1.51, 1.56, 1.62 and 1.50, respectively. The creatinine levels were statistically ($p<0.05$) similar between different groups.

The plasma AST and ALT levels fluctuated during the entire feeding trial. The plasma AST levels (IU/L) fluctuated between 94.20±1.09 to 156.17±2.22, 93.33±1.13 to 157.54±0.83, 91.51±1.17 to 161.90±1.11 and 93.67±0.92 to 158.34±1.77 in control, HME, HMP and HMEMP group, respectively. The plasma ALT levels (IU/L) fluctuated between 36.60±1.17 to62.94±1.14, 35.07±1.12 to 63.08±1.28, 32.95±1.07 to 61.33±1.04, and 33.06±1.15 to 62.68±1.15 in in control, HME, HMP and HMEMP group, respectively. The difference in AST and ALT activity was found to be non-significant ($p<0.05$) in all the groups.

Overall mean values of total immunoglobulin (mg/ml) was 31.75 (control), 33.23 (HME), 36.11 (HMP), and 36.80 (HMEMP) mg/ml during pre-partum period. Statistically, total immunoglobin concentration was significantly ($p<0.05$) higher in HMP and HMEMP group followed by HME and control group.

The levels of SOD were significantly (P<0.05) higher in animals fed diets having 40% extra protein and 30% extra energy (HMEMP) or 30% extra energy (HME) and was least in animals fed either 40% extra protein (HMP) or control diets. The overall mean of SOD in control, HME, HMP and HMEMP group was 2375.87, 2576.49, 2368.32 and 2600.62, respectively.

Cortisol

The data related to Cortisol (ng/ml) levels in pregnant Murrah buffaloes fed different plane of nutrition has been presented in **Table 3**. With advancement of pregnancy, levels of cortisol increased and reached their maximum level in the last trimester of pregnancy. The cortisol levels ranged from 2.75 to 6.19 in control group, from 3.11 to 4.96 in HME group, from 3.18 to 7.38 in HMP group and from 3.09 to 5.25 in HMEMP group, respectively. Supplementation of extra energy and protein to pregnant Murrah buffaloes significantly ($p<0.05$) affected the cortisol. At parturition, levels of cortisol had increased and its concentration in blood was 9.13, 8.02, 9.98 and 8.56 in control, HME, HMP and HMEMP group, respectively. Murrah buffaloes have high cortisol levels at the time of parturition because of the high-stress condition in animals.

Haematological parameters

During pregnancy, a decrease in Hb content could be due to blood dilution which occurs as a consequence of increase in plasma volume (Singh et al, 1991). With advancing pregnancy, blood volume increased which resulted in dilution of blood constituents. However, RBC count was high in dams fed with 30% extra energy and 40% extra protein as compared to other treatment groups throughout the study period. Supplementation of extra nutrients in form of energy and protein increased the

Table 2: Effect of plane of nutrition on blood biochemical parameters of pregnant Murrah buffaloes

	Control	HME	HMP	HMEMP
Glucose (mg/dl)	56.67^{b}±0.9	61.04^{a}±0.89	58.25^{b}±0.94	61.25^{a}±1.01
Total protein (g/dL)	7.90^{b}±0.08	8.02^{b}±0.09	8.37^{a}±0.07	8.45^{a}±0.09
Albumin (g/dL)	3.10^{b}±0.04	3.19^{ab}±0.03	3.27^{a}±0.06	3.32^{a}±0.06
Blood urea nitrogen (mg/dL)	31.17^{bc}±0.88	30.09^{c}±0.96	35.65^{a}±1.08	33.39^{ab}±0.93
Creatinine (mg/dL)	1.51±0.058	1.56±0.083	1.62±0.078	1.50±0.077
SGOT/AST (U/L)	106.00±1.94	107.31±2.08	105.38±2.25	106.44±2.24
ALT (U/L)	48.75±1.65	47.31±1.77	45.72±1.78	46.57±1.82
Total immunoglobulin (mg/ml)	31.75^{b}±0.69	33.23^{b}±0.66	36.11^{a}±0.63	36.80^{a}±0.69
SOD (U/mg Hb)	2375.87^{b}±57.47	2576.49^{a}±66.5	2368.32^{b}±56.27	2600.62^{a}±70.25

[a,b,c,d] Means bearing different superscripts in a row differ significantly (P<0.05)

nutrient supply to bone marrow for erythropoietic activity and thus more RBC formation for high fetal oxygen demand. Similar to the present findings, Beutler et al. (1970) observed that dietary protein levels significantly affect hematopoiesis and for synthesis of globulin part of hemoglobin proteins are required. Shebaita et al. (1973) also reported that in cattle protein supplementation have significant impact on the blood parameters i.e. hemoglobin, PCV and red blood cells count, than the energy supplementation because the protein plays an important role in hematopoiesis even though other nutrients are also involved.

Earlier some workers reported a decrease in PCV with advancing pregnancy, and it is possibly due to an increase in RBC volume causing increased water volume in advanced pregnancy. (Roy et al. 2010). PCV (%) in the present finding was in range of earlier report of Mir et al (2008). The lower Hb and PCV in the groups fed control diets is an indication of decreased dietary protein availability as indicated by reduced nitrogen utilization by that group. Similar to the present findings a few authors also observed lowered Hb and hematocrit levels in sheep by feed restriction mainly for decreasing availability of dietary protein (Oetzel et al. 1988). It was suggested that severe deficiency of protein interferes with Hb production (Whitehair, 1958). In the present study, TLC was within range of earlier report of Pande et al. (2016). The mean (%) of lymphocytes, neutrophils, monocyte, eosinophils and basophil count was not affected the plane of nutrition and all the experimental groups has statistically similar count of above said parameters. In the present study mean values of lymphocytes, neutrophils, monocyte, eosinophils and basophil were in agreement with findings of Mir et al. (2008). However, Alhussien and Dang (2020) reviewed the stress and phagocytic cells/RBCs and found that it's complex and poorly understood. Though, the results from the experiment were presented in a similar manner.

Blood biochemical parameters

A number of factors like time of feeding, nutritive values of the diets, stress conditions, physiological status of the animal etc. affects the circulating glucose levels (Terzano et al. 2005) and its concentration is regulated by some hormones like insulin, cortisol, glucagon, somatotropin and adrenaline. Results of present study were in agreement of previous studies who found that *ad libitum* feeding of higher energy diets increased the blood glucose concentration in prepartum phase compared with restricted diets fed animals (Guo et al. 2007). Earlier Kalasariya et al. (2017) reported that supplementation of high protein concentrate had a favourable impact on increasing the plasma total protein values. The concentration of total protein, albumin and globulin were within physiological range of earlier report of Abd Ellah et al. (2013). Critical analysis of data showed that there was increase in BUN concentration from 1st month of study to the end of study period. The BUN serve as an indicator of protein metabolism and its utilization. BUN concentration is linearly and positively correlated to CP ingested (Huntington et al. 2001). In the rumen, extensive degradation of dietary CP produce NH_3, accumulates and surpass the requirement of microbes, absorbed by rumen wall into blood, carried into liver and converted to urea. Urea is then transported through blood to kidney to be excreted through urine, however some amount is recycled through rumen wall or saliva to digestive tract (Church, 1993). In the present study, higher concentration of BUN at higher dietary CP might be due to higher ruminal ammonia absorption, excessive quantity of ammonia detoxified in liver to urea. James et al. (1999) found that urea is formed in the liver when microbial degradation of dietary CP in the rumen is not incorporated into microbial protein, but absorbed, causing an elevation of BUN. The concentration of BUN and creatinine in the present study were within normal physiological changes during this period and are in close agreement with the findings of Abd Ellah et al. (2013). As the date of parturition comes near, there was decline in immunoglobulin concentration in four respective groups, indicating immune suppression on the day of calving. These variations are owed to normal physiological changes during this period and are in close agreement with the findings of Aggarwal et al. (2016). The results of present study were in accordance with earlier findings in gestation. Talvelkar et al. (2008) observed an increase in SGPT from early (53.98± 13.44) to mid gestation (71.83±10.24) and then lowest in last gestation (39.26±4.19). However, in adult buffaloes, Pizzuti and Salvatori (1993) reported higher ALT values ranging from 176 to 219 IU/L at different pre

Table 3: Effect of plane of nutrition on Cortisol (ng/ml) of pregnant Murrah buffaloes

Month	Control	HME	HMP	HMEMP
Zero day	2.75±0.19	3.11±0.11	3.18±0.13	3.09±0.20
1st	3.76^{b}±0.14	3.43^{b}±0.14	4.41^{a}±0.17	3.58^{b}±0.12
2nd	4.64^{a}±0.13	3.89^{b}±0.08	4.92^{a}±0.14	4.08^{b}±0.16
3rd	5.49^{b}±0.16	4.37^{c}±0.12	6.08^{a}±0.13	4.53^{c}±0.15
4th	6.19^{b}±0.18	4.96^{c}±0.14	7.38^{a}±0.11	5.25^{c}±0.15
Overall	5.02^{b}±0.16	4.16^{c}±0.11	5.70^{a}±0.20	4.36^{c}±0.12
At parturition	9.13^{b}±0.22	8.02^{c}±0.25	9.98^{a}±0.20	8.56^{bc}±0.24

^{a,b,c,d} Means bearing different superscripts in a row differ significantly (P<0.05) and post- partum interval. Boots et al. (1969) stated that during pregnancy alteration in progesterone and estrogen levels may change SGPT activity. During prepartum period, AST levels fluctuated between 123.22 ± 2.17 to 148.32 ± 0.71 and 125.40 ± 7.66 to148.37 ± 6.93 IU/L during prepartum in control and Vitamin E supplemented buffaloes respectively (Mili et al. 2015). Earlier increasing SGOT activity from early gestation (153.40 ± 6.09) to mid gestation (173.69 ± 14) has been reported in buffaloes which was followed by lowest in late gestation (Talvelkar et al. 2008). The concentration of plasma cortisol is a useful physiological marker of stress. In most of the animals it is a primary glucocorticoid, secreted by zona fasciculate of adrenal cortex and secreted within minutes after exposure of animal to some stressful conditions. The primary function of cortisol is to mobilise energy reserves to promote hyperglycaemia by stimulating hepatic gluconeogenesis and reducing cellular glucose uptake. In acute stress conditions, cortisol allows an individual to respond to stressors by supporting energy mobilization. According to previous studies (Larson, 2005) cortisol activity has been associated with muscle catabolism and nitrogen losses. The levels of cortisol were within range of earlier report of Aggarwal et al. (2016). Analysis of glutathione and enzymes (SOD, GPx and catalase) serves as important marker for oxidative stress and antioxidant status, respectively. Higher erythrocyte SOD activity suggests lower antioxidant status and higher oxidative stress (Gaál et al. 2006). During, the present study, the SOD activities were significantly higher in HMEMP group, which indicates higher stress management or alternatively reducing stress level on animals. The findings of present study were not in agreement with findings of Chaudhari et al (2018).

Conclusion

Supplementation of metabolizable energy and protein beyond standard dietary requirements during late pregnancy in Murrah buffaloes significantly improved key hematological and biochemical parameters, indicating enhanced physiological status and metabolic health. The increased red blood cell count, hemoglobin concentration, and packed cell volume in groups with higher protein and energy levels (HMP and HMEMP) indicate an improved oxygen-carrying capacity essential for fulfilling the heightened metabolic requirements of gestation. Buffaloes fed diets enriched with both energy and protein exhibited significantly increased plasma glucose and total protein concentrations, indicating enhanced nutritional status and efficient nutrient utilization. The increase in blood urea nitrogen (BUN) levels in high-protein groups did not negatively affect renal function, as evidenced by stable creatinine concentrations. Additionally, total immunoglobulin concentrations were elevated in the supplemented groups, indicating sustained or improved immune competence. The activities of hepatic enzymes (ALT and AST) remained unchanged by dietary treatments, suggesting the absence of hepatotoxic effects. Cortisol levels were significantly lower in buffaloes provided with additional dietary energy (HME and HMEMP), indicating a potential reduction in physiological stress during late pregnancy. Increased superoxide dismutase (SOD) activity in energy-supplemented groups indicates enhanced antioxidant defense, which may reduce oxidative stress linked to late gestation. The findings indicate that providing Murrah buffaloes with enhanced nutrition during late pregnancy improves hematological profiles, metabolic function, and stress modulation, thereby positively affecting fetal development and preparing the dam for effective lactation. The implementation of nutritional strategies has the potential to improve reproductive efficiency and calf vitality in tropical buffalo production systems.

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Conflict of Interest The authors do not have any conflict of interest.

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