***Original Research Article***

 **Haemato-biochemical profile of Indigenous Nattukuttai breed calves fed with varying levels of energy and protein**

**ABSTRACT**

**Aim:** The study was undertaken to evaluate the haemato-biochemical profile of indigenous Nattukuttai breed calves fed with varying levels of energy and protein.

**Study design**: Completely Randomized Design

**Place and duration of study:** The study was conducted at Conservation Centre for Nattukuttai cattle, Post graduate Research Institute in Animal Sciences, Kattupakkam, Tamil Nadu Veterinary and Animal Sciences University for a period of 6 months from August 2024 to February 2025.

**Methodology:** Twenty Nattukuttai calves were divided into five treatment groups fed with varying levels of energy and protein at 100%, 110%, 90% and 80 % of ICAR (2013) recommendations for T2, T3, T4 and T5, respectively and in T1 groups the experimental calves were maintained only on grazing without any additional supplementations. At the end of the experimental trial, 5 ml of blood samples from each calves were collected.

**Results:** The values of various haematological parameters (RBC, WBC, lymphocytes, monocytes, granulocytes, haemoglobin, haematocrit, MCV, MCH and MCHC) and blood metabolites (blood glucose, total plasma proteins, albumin, globulin, BUN, creatinine, Ca, P, triglycerides and cholesterol) were within normal physiological range.

**Conclusion:** It can be inferred from the results that the ration with varying levels of energy and protein did not alter the haemato-biochemical profile of Nattukuttai breed calves.

***Key words:*** *Dietary energy and protein levels, Haematology, Nattukuttai breed calves, Serum biochemical profile.*

**1.INTRODUCTION**

India has diversified animal genetic resources with 53 well recognized indigenous cattle breeds (ICAR-NBAGR report, 2025) which are well adopted for different agro-climatic regions of the country. According to 20th livestock census, the population of indigenous and non-descript cattle decreased by 6 per cent. The preservation and conservation of the indigenous germplasm has gained priority in the recent years and several attempts were being made to improve the performance of indigenous breeds (Sripad *et al*., 2014). Nattukuttai is a unique short stature, non-descriptive cattle possess distinct biometrical attributes. They are predominantly reared by farmers belonging to North eastern agro-climatic zone of Tamil Nadu and plays a major role in sustaining the livelihood of cattle rearers in its breeding tract (Vinothkumar, 2014). These cattle are well adopted to local agro-climatic conditions and mostly reared under zero input system solely on grazing and open housing systems. Globally, in dairy production systems, inadequate nutrition has been emphasized as the major constraint (Devendra, 2000).

Providing an optimal level of nutrients in the diet of livestock is considered as utmost importance for their growth, production, and reproduction. Proteins and energy are indeed the most critical nutrients influencing calf growth. Blood serves a number of functions in the body and any alteration in the constituents of the blood reflects the functional status of an animal, hence haematological and biochemical profile are considered as important bioindicators of the nutritional status of an animal. Animal diet must be supplemented with adequate protein and energy to maintain normal biochemical profile. Analysing blood biochemical status along the growth data provides valuable insights into the synchronized effects of dietary energy and protein on their growth and development (Sharma *et al*., 2016). Therefore, the present study was designed to investigate the effect of varying levels of energy and protein on haematological and serum biochemical parameters in Nattukuttai calves.

**2.MATERIALS AND METHODS**

**2.1 Animals and experimental design**

The experiment was conducted at Conservation Centre for Nattukuttai cattle, Post graduate Research Institute in Animal Sciences, Kattupakkam, Tamil Nadu Veterinary and Animal Sciences University. Twenty Nattukuttai calves of 2 to 3 months age and uniform body weight were divided into 5 groups of 4 animals each in such a way that their average body weights (20.01± 1.61 kg) were almost similar. The experimental rations were formulated with different energy and protein levels in order to provide 100, 110, 90 and 80 % of ICAR (2013) requirements in groups T2, T3, T4 and T5, respectively. T1 group calves maintained only on grazing as in farmers field conditions which also serves as control. Ingredient composition of experimental rations were presented in Table 1. Experimental rations were mixed fortnightly and the animals were fed calculated amount of concentrate and roughages for a daily gain of 200g adjusted thereafter according to changes in body weights. The experimental trial was conducted for a period of 6 months to arrive optimal levels of nutrients from 3 months to 9 months of age.

**Table 1. Ingredient composition of experimental diet fed to Nattukuttai breed calves**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ingredients** | **T1 (Control)** | **T2 (100%)** | **T3 (110%)** |  **T4 (90%)** | **T5 (80%)** |
| Maize | GRAZING    | 28.00 | 45.85 | 12.00 | 0.00 |
| Groundnut oil cake | 17.85 | 22.05 | 16.20 | 15.30 |
| De-oiled rice bran | 22.05 | 0.00 | 29.70 | 22.60 |
| Mineral mixture | 1.40 | 1.40 | 1.40 | 1.40 |
| Salt | 0.70 | 0.70 | 0.70 | 0.70 |
| Hybrid Napier (CO4) grass | 18.26 | 18.26 | 21.33 | 32.00 |
| Paddy straw | 11.74 | 11.74 | 18.67 | 28.00 |
| Total |   | 100 | 100 | 100 | 100 |

**2.2 Blood sampling and analysis**

About 5 ml of blood samples were collected in heparinised vacutainers at the end of the experimental trial from each animal by jugular puncture. Haematological parameters were studied immediately in fresh blood sample using auto-haematological analyser (Mindray, model BC-2800). The haematological examinations were performed within 24 hours of sampling to avoid errors due to cell lysis. For the estimation of serum biochemical parameters, blood samples collected in EDTA coated vacutainer and centrifuged at 3000 rpm for 15 minutes to separate plasma and stored at -200 C until further analysis. Serum total protein, albumin, glucose, blood urea nitrogen (BUN), creatinine, ALT (Alanine transaminase), AST (Aspartate transaminase), cholesterol, triglycerides, calcium and phosphorous were analysed by standard commercial kits using A15 biosystem Automatic Biochemical Analyzer (Biosystem, S.A. Spain) as per the guidelines provided. Globulin content (g/dl) was determined by subtracting albumin content from the total protein content. Statistical analysis of data was done using one way ANOVA (Snedecor and Cochran, 2007) and suitable superscripts were attributed to mean values for statistical significance.

**3.RESULTS AND DISCUSSION**

The haematological and serum biochemical profile of the Nattukuttai calves was assessed to explore the effect of variation in dietary energy and protein levels.

**3.1 Haematological parameters**

The mean values of various haematological parameters (RBC count, WBC count, lymphocytes, monocytes, granulocytes, haemoglobin (Hb), haematocrit, MCV, MCH, MCHC) were presented Table 2. The results indicated that all haematological parameters (RBC count, WBC count, lymphocytes, monocytes, granulocytes, haemoglobin (Hb), haematocrit, MCV, MCH, MCHC) were in the normal physiological range in all the treatment groups and also indicating that different dietary energy and protein levels did not affect these parameters to any significant extent. Normal physiological range of RBCs, Hb, MCV, MCH and MCHC indicates normal erythropoiesis in experimental calves. Similarly, Ajay Singh and Bandla Srinivas (2020) observed that except haemoglobin and platelet other haematological parameters (RBC count, WBC count, PCV, Neutrophils, Lymphocytes and Monocytes) were non-significant in Deoni calves when fed with recommended plane and 25 % higher plane of nutrition. Daneshvar *et al.* (2017) reported that different levels of milk feeding and crude protein levels in calf starter ration did not alter haematological parameters such as Hb, PCV, RBC and WBC count. Kumar *et al.* (2013) observed that when Sahiwal calves were fed on isocaloric diets containing different protein levels of 15, 13.5 and 16.5% in groups T1, T2 and T3, respectively, haematological parameters (haemoglobin, haematocrit, MCV, MCH, MCHC, RBC count, WBC count, granulocytes, lymphocytes and monocytes) were similar in all treatment groups and were within normal physiological range. In contrast to the present findings, Sharma *et al.* (2020) stated that total leucocyte count was decreased significantly (p=0.05) as the protein level increased in calf starters. In the present study, Hb values varied from 9.03 to 9.93 g/dl across different treatment groups which were within the normal physiological range (8.0-15 g/dL) as reported by Kaneko *et al.* (2008).

**Table 2. Effect of varying levels of energy and protein on haematological parameters in Nattukuttai calves (Mean\* ± SE)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **T1 (Control)** | **T2 (100%)** | **T3 (110%)** |  **T4 (90%)** | **T5 (80%)** |
| RBC (X106/µl) | 7.45 ± 1.12 | 7.88 ± 0.52 | 8.13 ± 0.48 | 8.05 ± 0.24 | 7.39 ± 0.47 |
| WBC (X103/µl) | 11.36 ± 0.90 | 12.05 ± 0.76 | 11.08 ± 1.03 | 11.33 ± 1.34 | 11.63 ± 1.01 |
| Lymphocyte (%) | 62.26 ± 1.91b | 60.35 ± 2.13ab | 58.28 ± 1.31a | 58.03 ± 3.27a | 57.08 ± 2.50a |
| Monocyte (%) | 7.63 ± 1.30a | 9.83 ± 0.53b | 9.73 ± 0.91b | 9.53 ± 1.11b | 9.63 ± 1.89b |
| Granulocyte (%) | 30.13 ± 0.38a | 30.08 ± 1.97a | 34.5 ± 0.76b | 32.45 ± 0.66b | 33.30 ± 0.98ab |
| Haemoglobin (g/dl) | 9.03 ± 0.90 | 9.63 ± 0.53 | 9.8 ± 0.75 | 9.93 ± 0.36 | 9.53 ± 0.86 |
| Haematocrit (%) | 23.23 ± 2.08a | 25.05 ± 1.59ab | 26.03 ± 1.94b | 27.28 ± 1.15b | 25.33 ± 1.50ab |
| MCV (fL) | 31.53 ± 1.86a | 31.88 ± 2.14ab | 35.13 ± 1.44c | 33.90 ± 1.34ab | 34.35 ± 1.50bc |
| MCH (pg) | 12.17 ± 0.58 | 12.2 ± 0.73 | 12.9 ± 1.20 | 12.6 ± 0.43 | 12.83 ± 0.51 |
| MCHC (g/dl) | 38.8 ± 0.51c | 38.38 ± 0.34bc | 36.93 ± 0.60a | 37.35 ± 1.23ab | 37.55 ± 0.56ab |

\*Mean of four replications

Means bearing different alphabets as superscripts in the same row differ significantly (p=0.05)

**3.2 Serum biochemical parameters**

The mean values of various blood metabolites (Total plasma proteins, albumin, globulin, glucose, blood urea nitrogen (BUN), creatinine, ALT and AST) were presented in Table 3. The mean total plasma protein concentration was significantly (p=0.05) higher in T2 (100%) and T3 (110%) groups compared to T1, T4 and T5 groups might be due higher energy and protein level in the diet. Total plasma protein level in serum reflects availability of protein and their low level indicates dietary protein deficiency (Sahoo *et al.,* 2009). There was positive correlation between total plasma protein and CP (+0.276), DCP (+0.245), MP (+0.279) and ME (+0.236) intake (Sahoo *et al.,* 2009). Chang *et al.* (2013) also observed that restricting the protein intake than optimum levels in beef calves resulted in lower plasma protein concentration. Contrary to this, no effect of different crude protein levels (100, 90 and 80% of Kearl, 1982 recommendations) was observed on blood protein concentration (5.62 to 6.49 mg/dl) in Murrah males (Verma *et al.,* 2009). The overall mean values of albumin and globulin concentration across the treatment groups were within normal physiological range. The total glucose concentration was significantly (p=0.05) higher in T3 groups might be due to diet supplemented with higher energy and protein levels (110% of ICAR recommendations). Similarly, Verma *et al.* (2009) also reported higher glucose in growing Murrah bulls when fed with diet as per Kearl, (1982) recommendations. Similar results of high blood glucose in high protein, high energy group was also reported by Medhi *et al.* (2018) in growing yaks. Contrary to this, Singh *et al.* (2014) observed that dietary protein level had no effect on blood protein, urea and glucose in Bhadawari buffalo heifers when fed on standard protein group, low protein group (20% less protein) and high protein group (20% more protein) than ICAR recommendations.

In the present study, the BUN concentration was significantly (p=0.05) higher in all treatment groups compared to T1. Similarly, Prusty *et al.* (2022) reported a positive relationship between dietary protein and plasma urea nitrogen concentration in Murrah buffalo calves which support present findings. Contrary to this, Lohakare *et al.* (2006) reported that when three iso-caloric diets at different CP levels (75%, 100% and 125% of Kearl, 1982 CP recommendations) were fed to crossbred calves no significant effect of protein levels on serum urea concentration was observed and serum levels of urea was within normal range. Similarly, Niranjan *et al.* (2017) reported blood glucose and ammonia-N did not differ significantly among the treatments with varying level of energy and protein. Kumar *et al.* (2013) also observed that the dietary protein levels did not have any significant effect on the blood glucose and BUN level in growing Sahiwal calves. The mean values of creatinine concentration were similar in all the treatments groups. The overall mean values of ALT and AST was significantly (p=0.05) higher in treatment groups (T2, T3, T4 and T5) compared to control (T1). Contrary to this Mondal *et al.* (2021) observed that varying levels of metabolizable protein and metabolizable energy viz. 100,100; 115,100; 100,115; 115,115% as per ICAR (2013) recommendations did not influence plasma ALT and AST activity in buffalo calves.

The Ca and P levels were significantly (p=0.05) lower in T1 (only grazing) and T5 (80 % of ICAR recommendations) groups however it was within normal physiological range indicating that diet was sufficient to maintain normal serum Ca and P levels. Similarly, Lohakare *et al.* (2006) also observed no significant difference in serum calcium and phosphorus level in five months old male crossbred calves fed with different protein levels (100, 75 and 125 per cent of protein requirement). The triglycerides were significantly (p=0.05) lower in T4 and T5 groups and cholesterol contents were significantly (p=0.05) lower in T5 (80 % of ICAR recommendations) groups might be due to more roughages in their diet. T3 group had higher triglycerides and cholesterol level compared to other groups as in this group the calves were fed energy and protein dense diet (110 % of ICAR recommendations). Similarly, significantly (p˂0.05) higher cholesterol levels were observed in Holstein Kankrej heifers on the feeding of high energy and high protein diet (Hadiya *et al.,* 2019).

**Table 3. Effect of varying levels of dietary energy and protein on serum biochemical parameters in Nattukuttai calves (Mean\* ± SE)**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Parameters** | **T1 (Control)** | **T2 (100%)** | **T3 (110%)** |  **T4 (90%)** | **T5 (80%)** |
| Total protein (g/dl) | 5.38 ± 0.57ab | 5.95 ± 0.31b | 6.23 ± 0.38b | 5.75 ± 0.77ab | 4.90 ± 0.55a |
| Albumin (g/dl) | 2.85 ± 0.33ab | 3.53 ± 0.30c | 3.48 ± 0.15c | 3.05 ± 0.54bc | 2.45 ± 0.31a |
| Globulin (g/dl) | 2.55 ± 0.31 | 2.43 ± 0.28 | 2.75 ± 0.33 | 2.70 ± 0.54 | 2.45 ± 0.83 |
| Glucose (mg/dl) | 80.25 ± 3.30a | 83.00 ± 2.45ab | 86.50 ± 2.38b | 82.50 ± 2.08a | 80.00 ± 1.63a |
| Creatinine (mM/L) | 0.58 ± 0.59ab | 0.79 ± 0.15b | 0.74 ± 0.20b | 0.78 ± 0.19b | 0.46 ± 0.12a |
| BUN (mg/dl) | 9.74 ± 0.44a | 14.54 ± 1.03b | 12.02 ± 2.02ab | 13.76 ± 1.11b | 12.77 ± 2.49b |
| ALT (U/L) | 23.00 ± 2.16a | 36.00 ± 2.71bc | 29.50 ± 3.11b | 37.50 ± 5.07c | 32.50 ± 6.45bc |
| AST (U/L) | 55.00 ± 2.83a | 62.50 ± 2.89b | 64.00 ± 2.58b | 64.75 ± 5.38b | 58.75 ± 4.35ab |
| Calcium (mg/dl) | 7.86 ± 0.94a | 8.58 ± 0.59ab | 8.11 ± 1.00a | 9.75 ± 0.84b | 7.85 ± 0.50a |
| Phosphorus (mg/dl) | 4.30 ± 1.20a | 7.05 ± 0.38b | 7.06 ± 1.14b | 7.43 ± 0.82b | 6.03 ± 0.37b |
| Triglycerides (mg/dl) | 150.50 ± 1.29b | 153.75 ± 2.63b | 155.25 ± 5.44b | 139.75 ± 4.57a | 144.50 ± 4.43a |
| Cholesterol (mg/dl) | 101.50 ± 4.20ab | 107.00 ± 3.65ab | 109.75 ± 6.99b | 102.25 ± 5.50ab | 98.50 ± 5.74a |

\*Mean of four replications

Means bearing different alphabets as superscripts in the same row differ significantly (p= 0.05)

**4. CONCLUSION**

The findings revealed that different haematological and biochemical constituents were within normal physiological range and it was unaltered by different dietary energy and protein levels in Nattukuttai calves. However, this data should be compared with feed intake and growth performance data to arrive optimum requirement levels for Nattukuttai calves.

**8. ETHICAL APPROVAL**

The experimental procedure was approved by the Institutional Animal Ethics Committee (IAEC) of Tamil Nadu Veterinary and Animal Sciences University (Approval No.48/DFBS/IAEC/2024 dated 10.04.2024).

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