***Original Research Article***

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

**ABSTRACT**

**Aim:** The study evaluated the haemato-biochemical profile of indigenous Nattukuttai calves fed varying energy and protein levels.

**Study design**: Completely Randomized Design

**Place and duration of study:** The trial was conducted over six months at the Conservation Centre for Nattukuttai Cattle, Post graduate Research Institute in Animal Sciences (TANUVAS, Kattupakkam).

**Methodology:** Twenty calves were allocated to five groups: T1 (grazing-only, control), T2 (100% ICAR 2013 recommendations), T3 (110%), T4 (90%), and T5 (80%). Blood samples (5 mL/calf) were collected via jugular venipuncture at trial end.

**Results:** Haemato-biochemical parameters (RBC, WBC, lymphocytes, monocytes, granulocytes, haemoglobin, haematocrit, MCV, MCH, MCHC, blood glucose, total plasma proteins, albumin, globulin, BUN, creatinine, Ca, P, triglycerides and cholesterol) were analysed and found to remain within established normal ranges for the breed, with no significant differences (p>0.05) across groups.

**Conclusion:** Results indicate that energy/protein variations within the tested levels did not alter the haemato-biochemical profile of Nattukuttai calves

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

**1.INTRODUCTION**

India possesses a rich diversity of animal genetic resources, with 53 well-recognized indigenous cattle breeds, as reported by ICAR-NBAGR (2025). These breeds are well adapted to various agro-climatic regions across the country. However, according to the 20th Livestock Census, the population of indigenous and non-descript cattle has declined by 6%. In recent years, the preservation and conservation of indigenous germplasm have become a national priority, with several initiatives aimed at improving the performance of native breeds (Sripad *et al.,* 2014). One such breed is the *Nattukuttai*, a unique, short-statured, non-descript cattle type with distinct biometrical characteristics. These animals are predominantly reared by farmers in the northeastern agro-climatic zone of Tamil Nadu and play a vital role in sustaining the livelihoods of cattle rearers within their breeding tract (Vinothkumar, 2014). Well adapted to local environmental conditions, *Nattukuttai* cattle are typically maintained under a zero-input system, relying solely on grazing and open housing.

Globally, inadequate nutrition remains a major constraint in dairy production systems (Devendra, 2000). Ensuring an optimal level of nutrients in livestock diets is essential for their growth, productivity, and reproduction. Protein and energy are particularly critical nutrients influencing calf development. Blood plays multiple physiological roles, and any alteration in its composition can reflect an animal’s functional and nutritional status. Therefore, haematological and biochemical profiles serve as important bioindicators of nutritional health. To maintain a normal biochemical profile, livestock diets must be adequately supplemented with protein and energy. Analysing blood biochemical parameters alongside growth data provides valuable insight into the synergistic effects of dietary energy and protein on calf growth and development (Sharma *et al.,* 2016). Accordingly, the present study was undertaken to evaluate the effect of varying levels of dietary energy and protein on the haematological and serum biochemical parameters of *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 prepared fortnightly and the animals were fed calculated amount of concentrate and roughages to support a daily weight 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 venipuncture. 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 serum 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, including RBC count, WBC count, lymphocytes, monocytes, granulocytes, haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC), are presented in Table 2. The results indicated that all haematological parameters remained within the normal physiological range across all treatment groups, suggesting that varying dietary energy and protein levels had no significant effect on these indices. The normal values of RBCs, Hb, MCV, MCH, and MCHC indicate normal erythropoietic activity in the experimental calves.

Similar findings were reported by Ajay Singh and Bandla Srinivas (2020), who observed that, except for haemoglobin and platelet counts, other haematological parameters (RBC count, WBC count, packed cell volume [PCV], neutrophils, lymphocytes, and monocytes) were not significantly affected in Deoni calves fed with recommended and 25% higher nutritional planes. Daneshvar *et al.* (2017) also reported that different levels of milk feeding and crude protein in calf starter diets did not significantly alter haematological parameters such as Hb, PCV, RBC count, and WBC count.

Furthermore, Kumar *et al.* (2013) found that Sahiwal calves fed isocaloric diets with varying protein levels (15%, 13.5%, and 16.5% in groups T1, T2, and T3, respectively) showed no significant differences in haematological parameters (Hb, haematocrit, MCV, MCH, MCHC, RBC count, WBC count, granulocytes, lymphocytes, and monocytes), with all values remaining within normal ranges.

In contrast to the present findings, Sharma *et al.* (2020) reported a significant decrease (p = 0.05) in total leukocyte count with increasing protein levels in calf starter diets. In the present study, Hb values ranged from 9.03 to 9.93 g/dL across the treatment groups, which falls within the normal physiological range of 8.0–15.0 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, including total plasma proteins, albumin, globulin, glucose, blood urea nitrogen (BUN), creatinine, alanine transaminase (ALT), and aspartate transaminase (AST), are presented in Table 3. The total plasma protein concentration was significantly (p = 0.05) higher in groups T2 (100%) and T3 (110%) compared to T1, T4, and T5, which may be attributed to the higher levels of dietary energy and protein provided in those groups. Total plasma protein concentration reflects protein availability in the diet, and lower levels may indicate dietary protein deficiency (Sahoo *et al.,* 2009). A positive correlation was observed between total plasma protein and intake of crude protein (CP) (+0.276), digestible crude protein (DCP) (+0.245), metabolizable protein (MP) (+0.279), and metabolizable energy (ME) (+0.236) (Sahoo *et al.,* 2009). Chang *et al.* (2013) also reported that restricting protein intake below optimal levels in beef calves led to reduced plasma protein concentrations.

The overall mean concentrations of albumin and globulin across the treatment groups were within normal physiological ranges. Glucose concentration was significantly (p = 0.05) higher in the T3 group, likely due to the higher energy and protein intake (110% of ICAR recommendations). Similar findings were reported by Verma *et al.* (2009) in growing Murrah bulls and by Medhi *et al.* (2018) in growing yaks. However, Singh *et al.* (2014) found that dietary protein levels had no significant effect on blood protein, urea, or glucose concentrations in Bhadawari buffalo heifers fed standard, low (–20%), and high (+20%) protein diets.

In the present study, BUN concentrations were significantly (p = 0.05) higher in all treatment groups compared to the control group (T1). This aligns with findings by Prusty *et al.* (2022), who reported a positive relationship between dietary protein intake and plasma urea nitrogen levels in Murrah buffalo calves. In contrast, Lohakare *et al.* (2006) reported no significant effect of varying dietary protein levels (75%, 100%, and 125% of Kearl, 1982 CP recommendations) on serum urea levels in crossbred calves, with all values remaining within the normal range. Similarly, Niranjan *et al.* (2017) and Kumar *et al.* (2013) found no significant differences in blood glucose and BUN levels among calves fed varying energy and protein levels.

The mean creatinine concentrations were similar across all treatment groups. However, ALT and AST activities were significantly (p = 0.05) higher in treatment groups T2, T3, T4, and T5 compared to the control group (T1). In contrast, Mondal *et al.* (2021) observed no significant changes in plasma ALT and AST activities in buffalo calves fed varying levels of metabolizable protein and energy (100:100, 115:100, 100:115, and 115:115% of ICAR, 2013 recommendations).

Calcium and phosphorus levels were significantly (p = 0.05) lower in T1 (grazing only) and T5 (80% of ICAR recommendations), though all values remained within normal physiological ranges, indicating that the diets were adequate for maintaining normal serum Ca and P levels. Similarly, Lohakare *et al.* (2006) reported no significant differences in serum calcium and phosphorus levels in five-month-old crossbred male calves fed with different protein levels (100%, 75%, and 125%).

Triglyceride levels were significantly (p = 0.05) lower in T4 and T5 groups, while cholesterol levels were significantly (p = 0.05) lower in the T5 group, which may be attributed to the higher roughage content in their diets. In contrast, the T3 group, which received a diet dense in energy and protein (110% of ICAR recommendations), exhibited higher levels of triglycerides and cholesterol. Similar findings were reported by Hadiya *et al.* (2019), who observed significantly (p < 0.05) higher cholesterol levels in Holstein × Kankrej heifers fed high-energy, high-protein diets.

**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).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, manuscript.

**9. REFERENCES**

Ajay Singh & Bandla Srinivas, (2020). Plasticity of gut and metabolic limitations of Deoni calves in comparison to crossbred calves on a high plane of nutrition. *Tropical Animal Health and Production*. <https://doi.org/10.1007/s11250-020-02368-6>.

Chang, S.S., Lohakare, J.D., Singh, N.K., Kwon, E.G., Nejad, J.G., Sung, K.I. & Hong, S.K. (2013). Limiting concentrate during growing period affect performance and gene expression of hepatic gluconeogenic enzymes and visfatin in Korean native beef calves. *Asian-Australasian Journal of Animal Sciences.*26:202–210.

Daneshvar, D., Khorvash, M., Ghasemi, E. & Mahdavi, A. H. (2017). Combination effects of milk feeding methods and starter crude protein concentration: Evaluation on performance and health of Holstein male calves. *Animal Feed Science and Technology*, 223: 1-12.

Devendra C, (2000). Strategies for improved feed utilization and ruminant production systems in the Asian region. *Asian Aust J. Anim.* Sci.13: 51–58.

Hadiya, K. K., Dhami, A. J., Chaudhari, D. V. and Lunagariya, P. M. (2019). Effect of high plane of nutrition on blood biochemical profile and onset of puberty in prepubertal HF X Kankrej crossbred heifers. *Indian Journal of Veterinary Science and Biotechnology*, 15(2), 14-17.

ICAR, 2013. Nutrient Requirements of Cattle and Buffalo. Indian Council of Agricultural Research, New Delhi.

Kaneko, J.J., Harvey, J.W. & Bruss, M.L. (2008). Clinical biochemistry of domestic animals. (6th Ed.). Elsevier Academic Press, USA, 916p.

Kearl, L.C. (1982). Nutrient requirements of ruminants in developing countries. Int. feedstuffs Institute, Utah Agril. Expt. Station, Utah State Uni. Logan, Utah, USA.

Kumar. D., Chander Datt, L.K. Das & Kundu, S.S. (2013). Effect of Different Dietary Protein Levels on Feed Intake and Blood Parameters Profile in Growing Sahiwal Calves. *Indian J. Anim. Nutr*. 30 (4): 370-373.

Lohakare, J. D., Pattanaik, A. K. & Khan, S. A. (2006). Effect of dietary protein levels on the performance, nutrient balances, metabolic profile and thyroid hormones of crossbred calves. *Asian-Australasian Journal of Animal Sciences*, 19(11): 1588-1596.

Medhi, D., Santra. A, Paul. A & Deb, S.M. (2018). Effect of plane of nutrition on blood biochemical parameters and attainment of sexual maturity in growing yaks. *Indian Journal of Animal Sciences,* 88 (4): 470–473.

Mondal, G., Papori talukdar, Tapan kumar das, Mukesh bhakat & Madhu Mohini, 2021. Influence of metabolizable energy and protein levels on age and weight at puberty in male buffalo. *Indian Journal of Animal Sciences,* 91 (6): 487–491.

NBAGR, ICAR-National Bureau of Animal Genetic Resources (NBAGR), Karnal, Haryana. 2025.

Niranjan, S K, Mondal G, Thakur M, Bhakat M, Tyagi N & Mohini M. (2017). Impact of metabolizable protein and energy levels on nutrients intake, growth and blood biochemical parameters in growing male buffalo. *Indian Journal of Animal Nutrition*, 34: 21–27

Prusty, S., Kundu, S. S. Sharma, V. K. & Mondal, G. (2022). Studies on Growing Murrah Buffalo Fed on Diverse Energy and Protein Ration and their Effect on Biochemical Parameters. *Indian J. Anim. Nutr.* 39 (1): 12-22

Sahoo, A., Pattanaik, A.K. and Goswami, T.K. (2009). Immuno-biochemical status of sheep exposed to periods of experimental protein deficit and realimentation. *Journal of Animal Science*.87: 2664-2673.

Sharma, V.K., Kundu, S.S., Prusty, S., Datt, C. & Kumar, M. (2016). Nutrient utilization, growth performance and blood metabolites in Murrah buffalo calves (Bubalus bubalis) divergently selected for residual feed intake. *Archives of Animal Nutrition*. 70: 455-469.

Sharma, B., Nimje, P., Tomar, S. K., Dey, D., Mondal, S. & Kundu, S. S. (2020). Effect of different fat and protein levels in calf ration on performance of Sahiwal calves. *Asian-Australasian Journal of Animal Sciences*, 33(1): 53-60.

Singh, S., Kushwaha, B.P., Maity, S.B., Singh, K.K. & Das, N. (2014). Effect of dietary protein on intake, nutrients utilization, nitrogen balance, blood metabolites, growth and puberty in growing Bhadawari buffalo (Bubalus bubalis) heifers. *Trop Anim Health Prod*. DOI 10.1007/s11250-014-0713-3.

Snedecor, G.W. & Cochran W.G. (2007). Statistical methods. Iowa State University Press, Ames Iowa, 8th edition.

Sripad, K., Kowalli, S. & Metri, R. (2014). Haematological profile of Khillar breed of cattle in Karnataka. *Veterinary World,* 7(5): 311-314.

Verma, A.K., Singh, P., Deshpande, K.Y., Verma, V & Mehra, U.R. (2009). Influence of dietary proteins levels on nutrient utilization and blood parameters in buffaloes fed on wheat straw based diets. *Animal Nutrition and Feed Technology*, 9: 21-28.

Vinothkumar, D. (2014). Evaluation of performance characteristics of a distinct cattle population (Nattukuttai Madu) in North- Eastern agro climatic zone of Tamil Nadu. M.V.Sc Thesis, Tamil Nadu Veterinary and Animal Sciences University, Chennai.