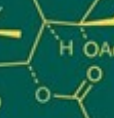
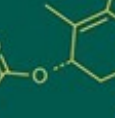
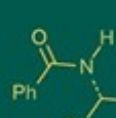
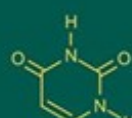


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Effect of dietary crude glycerol supplementation on haematological indices and blood biochemical profile of Deoni calves

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ABSTRACT

This study evaluated the effect of a crude glycerol-supplemented diet on the haematological profile and blood biochemical metabolites of indigenous Deoni calves. Ten calves with an initial live weight ranging from 133.88 ± 3.18 to 134.48 ± 2.45 kilograms were allotted uniformly, on the basis of body weight and age, into two dietary groups for a 60-day feeding trial. The control group was offered a basal diet comprising green fodder, ragi straw and concentrate in a roughage-to-concentrate ratio of 70:30, while the treatment group received the same basal diet with the concentrate fortified with 10 percent crude glycerol. Blood samples were collected on days 0, 30 and 60 for haematological and biochemical analysis. Red and white blood cell indices, serum creatinine, blood urea nitrogen, liver enzyme activities, total protein, albumin, glucose and triglyceride concentrations did not differ significantly between the control and treatment groups, although platelet count was significantly higher in glycerol-fed calves. Several parameters varied significantly across sampling periods, reflecting normal physiological and growth-related change rather than a treatment effect. These findings indicate that concentrate fortified with 10 percent crude glycerol does not adversely affect the haematological status or blood metabolic profile of growing Deoni calves, supporting the metabolic safety of crude glycerol as an alternative energy source in ruminant diets.

Keywords: Crude glycerol, Haematology, Blood biochemicals, Plasma metabolites, Deoni calves

1. Introduction

Rising demand for biofuel has destabilized the grain futures market and increased the cost of conventional livestock feed ingredients, prompting producers across the livestock sector to seek alternative and economical sources of dietary energy. Crude glycerol, a co-product of biodiesel processing, has emerged as one such alternative because of its increasing availability, comparatively low cost and a feed value reported to be comparable to that of maize when used as an energy source in ruminant rations (Hippen *et al.*, 2008) [1]. The United States Food and Drug Administration has reported that crude glycerol is a safe feed constituent for animal diets provided that impurities remain within acceptable limits (FDA, 2006) [2].

Dietary glycerol is extensively metabolized by the ruminal microflora, with propionate as the predominant end-product of its fermentation; the extent of ruminal glycerol degradation and the proportion escaping fermentation vary with the level of dietary supplementation and the method of administration (Kijora *et al.*, 1998) [3]. Feeding glycerol to transition dairy cows has been reported to alter selected blood metabolites and to influence lactation performance (DeFrain *et al.*, 2004) [4], while hepatic oxidation of the propionate arising from glycerol fermentation has been implicated in the regulation of feed intake in ruminants (Allen *et al.*, 2013) [5]. Blood haematological and biochemical indices are widely used as sensitive markers of the nutritional, metabolic and physiological status of an animal and are therefore useful for evaluating the metabolic safety of a novel feed ingredient such as crude glycerol.

Despite this body of work in sheep and dairy cattle, information on the haematological and blood biochemical response of indigenous zebu breeds such as Deoni calves to dietary crude glycerol remains limited. This study was therefore undertaken to evaluate the effect of feeding a crude glycerol-fortified concentrate, in combination with the basal diet, on the haematological profile and blood biochemical metabolites of growing indigenous Deoni calves.

2. Materials and Methods

2.1 Animals, treatments and experimental design

The study was conducted at the Livestock Research Centre, Southern Regional Station, ICAR-National Dairy Research Institute, Bengaluru, India. Ten Deoni calves with a uniform initial body weight of 133.88 ± 3.18 to 134.48 ± 2.45 kg were used in a 60-day feeding trial. Calves were allotted uniformly to one of two dietary treatments on the basis of body weight and age: a control group offered the basal diet only, and a glycerol group offered the basal diet with the concentrate fortified with an additional 10 percent crude glycerol per kilogram of concentrate. Nutrient requirements were calculated according to the guidelines of NRC (2001), found comparable to the recommendations of ICAR (2013). Maintenance requirements were calculated on the basis of mean body weight, and growth requirements were calculated on the basis of an average daily gain of 300 g/d.

All experimental protocols and animal-handling procedures were approved by the Institutional Animal Ethics Committee of ICAR-National Dairy Research Institute (IAEC approval No. CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2021/No. 7) and were conducted in accordance with the norms of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India.

Following a seven-day adaptation period, during which all animals received the control diet, calves in the treatment group were shifted to the control diet with the concentrate fortified with 10 percent glycerol. At the start of the experiment, animals weighed 133.88 ± 3.18 to 134.48 ± 2.45 kg and were 12 to 15 months of age. The feeding trial lasted eight weeks. The chemical composition of the experimental feed ingredients is presented in Table 1.

2.2 Blood sampling and analysis

Blood samples were collected on days 0, 30 and 60 of the feeding trial, prior to the morning feeding, from the jugular vein into 10 mL EDTA-coated vacutainer tubes. Plasma was separated by centrifugation at 3,000 rpm for 10 minutes and stored at -20°C until biochemical

analysis. Serum glucose and other blood metabolites were analysed using a GOD-POD kit (Span Diagnostics Ltd., India) and a UV-spectrophotometer (Labman Scientific Instruments Ltd., Chennai, India).

2.3 Statistical analysis

Data were analysed using the Statistical Package for Social Sciences (Version 16.0, IBM India Pvt. Ltd.). All values are expressed as mean $\pm$ standard error of the mean. Blood metabolite data were analysed using a completely randomized design (CRD), and treatment means were compared for significance at $p < 0.05$ to $p < 0.10$. It is noted that the trial used five calves per treatment group ($n = 5$); this sample size, while adequate for an initial 60-day trial, is modest, and the findings are best interpreted as a preliminary contribution ahead of larger confirmatory studies.

3. Results

3.1 Haematological profile

Red blood cell parameters, presented in Fig 1, varied significantly across sampling periods ($p < 0.05$), being highest in the first and third fortnights (8.38 and $8.53 \times 10^6/\mu\text{L}$) and lowest in the second fortnight ($7.97 \times 10^6/\mu\text{L}$), but did not differ significantly between the control and treatment groups or their interaction with period. Haemoglobin concentration was comparable between the treatment and control groups across periods. White blood cell count, also presented in Fig 1, ranged from 8.98 to $9.94 \times 10^3/\mu\text{L}$ and did not differ significantly between groups, periods or their interaction. Lymphocyte percentage, the dominant white cell fraction, was statistically comparable across all groups and periods, and granulocyte percentage, ranging from 2.76 to 3.54 percent, was similarly unaffected by treatment or period. Platelet count was approximately 18 percent higher in the treatment group ($391.73 \times 10^3/\mu\text{L}$) than in the control group and differed significantly between treatments ($p < 0.02$), although the treatment-by-period interaction was not significant; the overall period mean ranged from 325.30 to $385.90 \times 10^3/\mu\text{L}$.

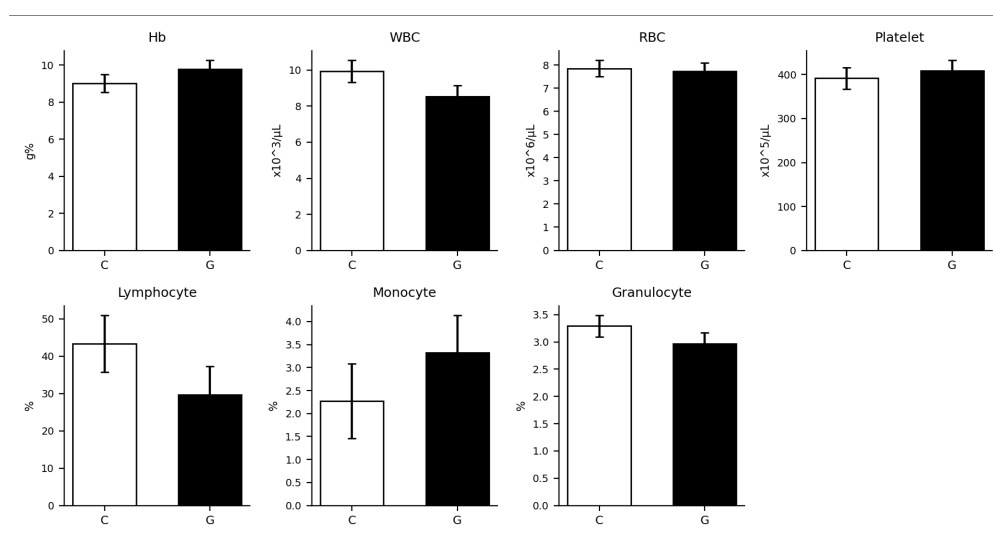


Figure 1. Haematological profile (overall treatment means $\pm$ SEM) of Deoni calves fed the control (C) or glycerol-supplemented (G) diet

3.2 Plasma metabolites

Serum creatinine, presented in Fig 2, was comparable between groups and their interaction with period but varied significantly with sampling period ($p < 0.001$), being 1.21 mg/dL at the start of the trial and 1.15 mg/dL by the third fortnight. Blood urea nitrogen differed significantly between the treatment-by-period interaction and between groups, averaging 13.73 mg/dL in the control group and 14.31 mg/dL in the treatment group. Serum alanine aminotransferase activity was comparable between the control (38.19 IU/L) and treatment (37.57 IU/L) groups, and values in both groups remained within the normal

physiological range. Aspartate aminotransferase activity, ranging from 87.32 to 103.40 IU/L across the pre- to post-experimental period, was also within the normal range but varied significantly with period ($p < 0.01$) and showed a weaker interaction with treatment ($p = 0.56$). Serum total protein ranged from 6.47 to 6.59 g/dL, with a mean albumin concentration of approximately 3 g/dL, and was comparable between groups and their periodic interaction. Blood glucose was comparable between the control and treatment groups ($p = 0.46$) and did not vary significantly with period, and triglyceride concentration, ranging from 39.36 to 43.94 mg/dL across the pre- to post-experimental period, was likewise unaffected by treatment.

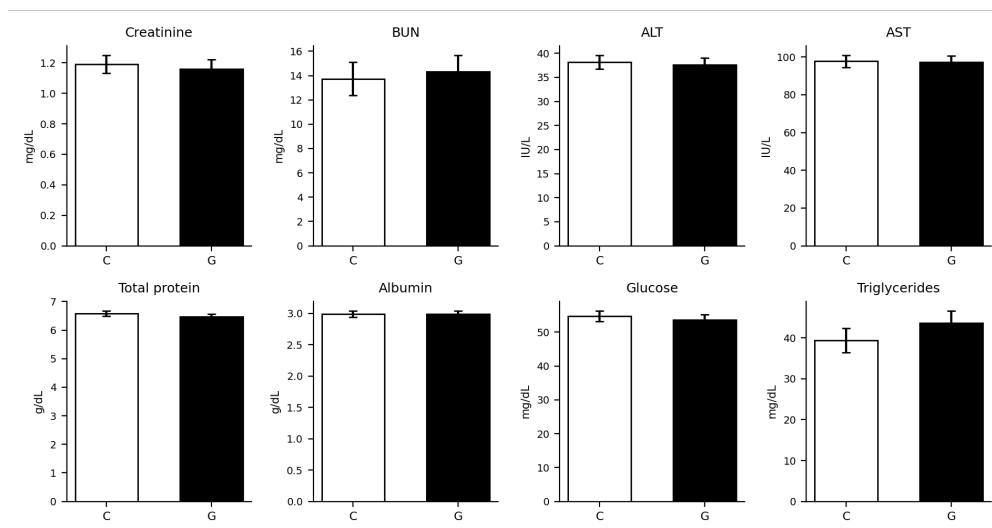


Figure 2. Plasma metabolite profile (overall treatment means $\pm$ SEM) of Deoni calves fed the control (C) or glycerol-supplemented (G) diet

4. Discussion

4.1 Haematological profile

High doses of glycerol have been reported to produce notable changes in red blood cell indices (Porcu *et al.*, 2020) [6]. The observed lymphocyte response is consistent with its role in immune defence and its regulation by multiple physiological factors (Roland *et al.*, 2014) [7], and the lack of a granulocyte response agrees with earlier reports that granulocyte and lymphocyte percentages are not affected by period or by glycerol-based dietary supplementation (Kang *et al.*, 2017) [8]. The platelet counts recorded here remained within the reported normal reference range of 160 to $800 \times 10^5/\mu\text{L}$ for cattle (Wood *et al.*, 2010) [9], indicating that despite the treatment-related increase, values stayed within a physiologically normal range.

4.2 Plasma metabolites

Plasma biochemical constituents are recognized indicators of the nutritional and physiological status of an animal (Xuan *et al.*, 2018) [10]. The blood urea nitrogen concentration recorded here, possibly reflecting the additional dietary energy supplied by glycerol, is comparable to the approximately 15 mg/dL previously reported in dairy cows (Rosler *et al.*, 1993) [11]. Elevated alanine aminotransferase activity is generally associated with hepatic injury, and the normal values recorded in both groups indicate normal liver function; seasonal, circadian and energy-status factors have been reported to influence alanine aminotransferase activity in dairy cows (Stojević *et al.*, 2005) [12], while aspartate aminotransferase is a recognized biomarker of hepatocellular health (Steen *et al.*, 1997) [13]. The total protein values recorded were within the physiological range but lower than the 9.80 to 10.40 mg/dL reported in lactating cows (Nozad *et al.*, 2012) [14].

4.3 Study limitations

It should also be noted that the present trial involved five calves per treatment group ($n = 5$). While this sample size is appropriate for an initial 60-day trial, it is modest relative to larger confirmatory studies, and international reviewers may reasonably scrutinize it. The consistent haematological and biochemical trends observed here nonetheless support this work as a valuable preliminary study, and larger-scale trials are recommended to confirm these findings.

5. Conclusion

Under the conditions of this study, dietary inclusion of crude glycerol at 10 percent of the concentrate mixture did not adversely affect the haematological profile or blood biochemical status of Deoni calves, with all measured indices remaining within normal physiological ranges except for a treatment-related increase in platelet count. These findings support the metabolic safety of crude glycerol as an alternative, non-conventional energy source in the diet of growing indigenous calves. Further studies of longer duration, larger sample size and across a range of inclusion levels are warranted to confirm these findings and to establish an optimum level of dietary glycerol inclusion for indigenous cattle breeds.

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Conflict of interest

The authors declare that there is no conflict of interest.

Tables

Table 1. Chemical composition of feed ingredients used in the experimental diet (% DM)

Item	Mix. Green fodder	Concentrate	Ragi Straw	Glycerol
DM	25.01	89.41	94.14	96.85
OM	91.33	87.16	91.70	99.84
CP	5.17	17.44	4.81	-
EE	1.49	1.91	2.82	-
TA	8.56	12.94	8.29	0.36
TCHO	50.21	67.66	84.07	-
NDF	66.04	45.03	73.41	-
ADF	43.43	11.76	47.17	-
ADL	5.04	3.85	3.38	-
NFC	18.69	22.60	10.66	99.64

DM = dry matter; OM = organic matter; CP = crude protein; EE = ether extract; TA = total ash; TCHO = total carbohydrate; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; NFC = non-fibre carbohydrate. Values are based on analysis of composite feed samples. The glycerol source contained approximately 96.85% dry matter. Hyphen (-) denotes a fraction not applicable/not determined for the glycerol ingredient, as crude glycerol is a non-fibrous, non-nitrogenous compound and was not analysed for crude protein, ether extract or fibre fractions.

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