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Effects of dietary crude glycerol supplementation on nutrient intake, digestibility and blood metabolites of crossbred calves

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Abstract

This study evaluated the effects of a crude glycerol-supplemented diet on nutrient intake, digestibility and blood metabolites in crossbred calves. Ten crossbred calves, with initial body weight ranging from 142.25 ± 4.81 to 142.59 ± 4.78 kilograms, were assigned uniformly to two groups for a 60-day feeding trial. The treatment diet contained 10% crude glycerol on a concentrate basis, while the control group received only the basal diet. No significant differences ($p > 0.05$) were observed between the treatment and control groups for feed intake or digestibility coefficients of dry matter, crude protein, acid detergent fibre and neutral detergent fibre. Likewise, mean serum glucose, aspartate aminotransferase and alanine aminotransferase concentrations were not affected ($p > 0.05$) by dietary treatment. Based on these findings, supplementation with crude glycerol at levels up to 10% of the concentrate mixture can be efficiently utilised in the diets of crossbred calves without adverse effects on intake, digestibility or blood metabolic profile, supporting its use as an alternative, non-edible-oil-derived energy source in ruminant production systems.

Keywords: Crude glycerol, nutrient digestibility, blood metabolites, crossbred calves, feed intake

1. Introduction

The rising cost of energy-rich feedstuffs has made alternative feed resources such as glycerol a major focus for the feed industry. The livestock feed industry has readily adapted such alternative ingredients to bridge the gap between the supply, demand and cost of energy-dense feed grains. Crude glycerol is a by-product of the non-edible oil-based biodiesel industry; for approximately every 100 litres of oil and 10 litres of methanol processed, about 100 litres of biodiesel and 10 litres of glycerol are generated ^[1]. The biodiesel industry is therefore likely to continue providing a substantial and increasing supply of crude glycerol that can be used as an alternative energy source in animal diets ^[2]. Supplementing dairy cow diets with crude glycerol has been reported to enhance the supply of glucose and improve metabolic status by serving as a substrate for gluconeogenesis, without adversely affecting feed intake ^[3, 4]. Other studies have found that feeding crude glycerol improved weight gain and feed efficiency in grazing Nellore bulls ^[5]. Furthermore, glycerol has shown the potential to improve feed digestibility in beef cattle and dairy cows, with inclusion levels of 10% to 20% of dietary dry matter used without adversely affecting the performance of lambs ^[6, 7, 8]. More recently, pre- and postpartum supplementation of a purified glycerol product has been shown to alter feeding behaviour and metabolic markers in transition dairy cows ^[9] while semi-refined glycerin has continued to be evaluated as

an alternative energy source for intake, digestibility and milk production in lactating cows ^[10]. Therefore, the present study was undertaken to test the hypothesis that inclusion of glycerol would affect feed intake, feed digestibility and blood metabolites when supplemented at an optimum level in the basal diet of crossbred calves.

2. Materials and Methods

2.1 Animals, treatments and experimental design

Ten crossbred calves were reared at the Livestock Research Centre unit, Southern Regional Station, ICAR-National Dairy Research Institute, Bengaluru, India. The calves were assigned uniformly to one of two dietary treatments based on body weight and age: a control group and a glycerol concentrate group. The treatment group received an additional 10% crude glycerol per kilogram of concentrate diet, while the control group received only the basal diet. A fixed level of glycerol inclusion was used as an additional energy source for the growing calves.

All experimental protocols and animal handling procedures were approved by the Institutional Animal Ethics Committee (IAEC) 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.

During a one-week adaptation period, all animals were fed the control diet (a mixture of concentrate, green fodder and ragi straw). The experiment began with animals weighing 142.25 ± 4.81 to 142.59 ± 4.78 kilograms body weight at 12 to 15 months of age. Calves were individually offered the experimental diet at a fixed proportion of dry matter intake relative to individual body weight, with daily intake recorded at 08:00 h and 17:30 h; residual roughage was weighed prior to the morning feeding. The feeding trial lasted eight weeks, and body weight was recorded at fortnightly intervals before the morning feeding. Faecal samples were collected manually during a seven-day digestibility trial, and both feed and faecal samples were oven-dried at 100 °C for 24 h at each sampling interval. The chemical composition of the experimental diets is presented in Table 1.

Table 1: Chemical composition of the crossbred calf diet with additional glycerol (% 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	-
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, 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.

2.2 Chemical analysis and calculations

The chemical composition of feed and faecal samples — dry matter, crude protein and ether extract — was determined according to standard AOAC procedures^[11], while neutral detergent fibre, acid detergent fibre and lignin were determined by the method of Van Soest *et al.*^[12]. Total digestible nutrients were calculated based on NRC guidelines^[13], and nutrient digestibility was estimated using the indirect digestibility method of Wallace and Van Dyne^[14], as follows:

Digestibility coefficient (%) = $[(\text{Total intake} - \text{Faecal output}) \div \text{Total intake}] \times 100$

2.3 Blood sampling and analysis

Blood samples were collected on days 0, 30 and 60 of the feeding trial, before 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 for biochemical analysis. Serum glucose and other blood metabolites were analysed using a GOD-POD kit (M/s Span Diagnostics Ltd., India) and a UV-spectrophotometer (M/s Labman Scientific Instruments Ltd., Chennai, India).

2.4 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. Feed intake, digestibility and blood metabolite data were analysed using a completely randomised design (CRD), and treatment means were compared for significance ranging from $p < 0.05$ to $p < 0.10$.

3. Results and Discussion

3.1 Intake and nutrient digestibility

The effects of the glycerol-supplemented diet on intake and digestibility of growing crossbred calves are presented in Figure 1. Overall mean feed intake — in terms of total dry matter, organic matter, crude protein, ether extract, neutral detergent fibre and acid detergent fibre intake — did not differ significantly between the experimental and control diets. Likewise, nutrient digestibility (dry matter, organic matter, crude protein, ether extract, neutral detergent fibre and acid detergent fibre) was not affected ($p > 0.05$) by glycerol inclusion. These results agree with Mach *et al.*^[7], who fed up to 12% glycerol to Holstein bulls, and with Gunn *et al.*^[2], who reported no change in dry matter intake with high-level glycerol-supplemented diets. Similarly, Chanjula *et al.*^[15] found no significant difference between glycerol-supplemented and control diets when glycerol replaced corn in goat diets, and Avila-Stagno *et al.*^[16] reported that crude glycerol supplementation of finishing lambs did not adversely affect nutrient intake or digestibility. Wang *et al.*^[6] reported increased dry matter digestibility with glycerol inclusion at 0% to 3.3% of dietary dry matter in cattle. In contrast, a decrease in dry matter intake was reported when a diet containing 10% glycerin replaced corn in feedlot steers^[17], and increasing glycerin to 4%, 8%, 12% and 16% of dietary dry matter reduced intake in finishing heifers^[18]. Dry matter intake has also been reported to decrease when glycerin was fed to finishing lambs at high inclusion levels of up to 45%^[19, 2].

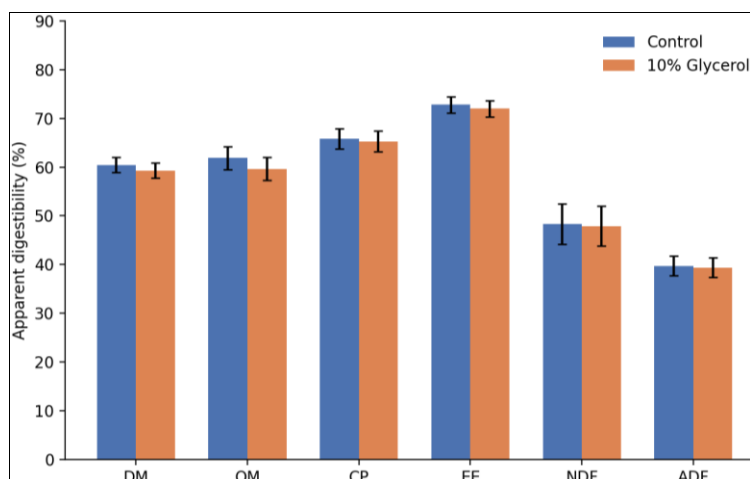


Fig 1: Apparent nutrient digestibility (%) of crossbred calves fed control and 10% crude glycerol diets

Hess *et al.* [20] reported that crude glycerol could be included at up to 15% of dietary dry matter in ruminant diets without negatively affecting dry matter or fibre digestibility. Taken together with the present findings, these data suggest that a reduction in dry matter intake may not occur until dietary glycerol concentrations exceed 20% of dry matter. Further research is warranted to determine the exact optimal inclusion level of glycerol in livestock diets.

3.2 Blood metabolites

Serum creatinine, blood urea nitrogen, total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glucose concentrations are presented in Figure 2. None of these parameters differed significantly ($p > 0.05$) between the control and glycerol-supplemented groups. Serum creatinine ranged from 1.00 to 1.05 mg/dL during the experimental period. Mapiye *et al.* [21] attributed reduced serum creatinine in indigenous Nguni cattle to their comparatively lower muscle mass relative to crossbred cattle; the mean creatinine values observed in the present study (1.00 to 1.05 mg/dL) were well below the upper reference limit of 1.5 mg/dL reported for cattle [22]. Hammond *et al.* [23] reported that blood urea nitrogen increased from 2.6 to 11.1 mg/dL as dietary crude protein increased from 6% to 18%, while glycerol supplementation has been reported to result in blood urea nitrogen concentrations of 11 to 15 mg/dL [24]. AST and ALT are

sensitive indicators of hepatocellular damage [25], and their plasma concentrations reflect hepatocyte status and can serve as biomarkers of liver health [26]. In the present study, ALT and AST concentrations were comparable between the control (33.80 and 94.11 IU/L, respectively) and glycerol-supplemented groups (34.40 and 94.41 IU/L, respectively), with the minor differences likely attributable to seasonal and physiological variation rather than dietary treatment [27]. Total protein was also similar between groups (6.59 to 6.75 g/dL), broadly comparable with values previously reported for high- and low-yielding dairy cows [28]. Blood glucose concentration did not differ significantly between groups ($p > 0.05$), although glucose tended to be numerically higher in calves fed the glycerol diet, consistent with previous reports of increased circulating glucose following glycerol feeding [4, 29]. In agreement with the present findings, Savitha and Punith Kumar [30] observed increased blood glucose with no change in serum creatinine, blood urea nitrogen or AST when glycerol was fed to Holstein-Friesian cows. The variation in glucose response to glycerol supplementation is likely influenced by the physiological state and energy balance of the animal [7]. Overall, the results of the present study indicate that crude glycerol can be included at up to 10% in a total mixed ration or glycerol-based diet without adversely affecting feed intake, digestibility, blood metabolites or the performance of crossbred calves.

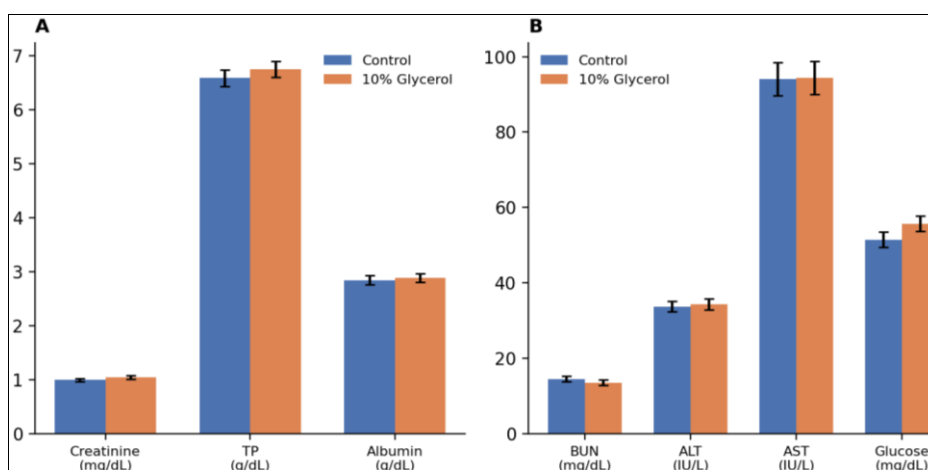


Fig 2: Blood metabolite concentrations in crossbred calves fed control and 10% crude glycerol diets

4. Conclusion

Crude glycerol proved to be a suitable alternative energy source in the diet of crossbred calves. Inclusion of up to 10% crude glycerol had no adverse effect on feed intake, nutrient digestibility or blood metabolite concentrations. Crude glycerol can therefore be effectively used as a partial energy source in the diets of crossbred calves, particularly where its inclusion improves overall diet energy density. Further long-term studies are recommended to evaluate the effects of higher inclusion levels of crude glycerol on the growth performance of crossbred calves and to determine the optimal feeding rate for this class of livestock.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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