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Effect of dietary crude glycerol supplementation on feed intake, nutrient utilization and digestibility in Deoni calves

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ABSTRACT

This study evaluated the effect of a crude glycerol-supplemented diet on feed intake and nutrient digestibility in 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. Body weight gain and dry matter intake increased steadily and comparably in both groups over the trial period. Nutrient intake and the digestibility of dry matter, organic matter, crude protein, ether extract, acid detergent fibre and neutral detergent fibre did not differ significantly between groups. These findings indicate that concentrate fortified with 10 percent crude glycerol can be included in the diet of growing Deoni calves without adversely affecting feed intake, growth or nutrient digestibility, supporting its potential as an alternative, non-conventional energy source for sustainable ruminant production systems.

Keywords: Crude glycerol, Feed intake, Nutrient utilization, Digestibility, 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 livestock feed and biodiesel processing, has emerged as one such alternative because of its increasing availability and comparatively low cost (Almeida *et al.*, 2017; Almeida *et al.*, 2019) [1, 2]. Glycerol has the potential to replace maize in ruminant diets and has been predicted to possess a feed value of 100 to 120 percent that of maize, making it a viable energy substitute (Hippen *et al.*, 2008) [3]. Crude glycerol is generated as a by-product of the base-catalysed transesterification of oils and fats used in the production of biodiesel (methyl and ethyl fatty acid esters) (He *et al.*, 2006) [4]. 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) [5].

With the increasing availability of glycerol as an energy source in ruminant rations, several studies have examined its effects on nutrient utilization. In sheep fed a low-starch concentrate diet, glycerol supplemented at 48, 78, 131 or 185 g/d (dry matter basis) did not affect the digestibility of organic matter, starch or cell-wall constituents (Südekum *et al.*, 2008) [6]. In contrast, supplementation with the same levels of glycerol in a high-starch concentrate diet decreased digestibility of cell-wall constituents and organic matter without affecting starch digestibility, and decreased the ruminal acetate-to-propionate ratio while increasing water intake, effects that may be of particular benefit to transition dairy cows (Südekum *et al.*, 2008) [6]. Additional studies have reported that glycerol supplementation improves rumen fermentation and feed efficiency (Hippen *et al.*, 2008) [3]. Dietary glycerol is extensively metabolized by the ruminal microflora, with propionate as the predominant end-product of its fermentation; however, 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; DeFrain *et al.*, 2004) [7, 8].

Despite this body of work in sheep and dairy cattle, information on the growth performance and nutrient utilization response of indigenous zebu breeds such as Deoni calves to dietary crude glycerol remains limited. This study was therefore undertaken to evaluate the effects of feeding a crude glycerol-fortified concentrate, in combination with the basal diet, on dry matter intake and nutrient digestibility in 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. 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 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.

Nutrient requirements of the calves were calculated according to the guidelines of NRC (2001), which were 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.

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

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. Feed intake and digestibility 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 Nutrient composition of the experimental diet

The chemical composition of the feed ingredients used in the trial is presented in Table 1. Mixed green fodder contained 25.01 percent dry matter and 5.17 percent crude protein, while ragi straw contained 94.14 percent dry matter and 4.81 percent crude protein. The concentrate, which contained 17.44 percent crude protein, supplied the additional nutrients required over and above the basal diet. Neutral detergent fibre content was 66.04, 45.03 and 73.41 percent, and acid detergent fibre content was 43.43, 11.76 and 47.17 percent, in mixed green fodder, concentrate and ragi straw, respectively. Ether extract content was 1.49, 1.91 and 2.82 percent in mixed green fodder, ragi straw and concentrate, respectively, and total ash content was 8.56, 12.94 and 8.29 percent in the same ingredients. Crude glycerol was included uniformly in the concentrate offered to the treatment group.

Following a seven-day adaptation period, during which all animals received the control diet (a mixture of concentrate, green fodder and ragi straw), 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. Calves were individually offered the experimental diet at a fixed proportion of dry matter intake relative to 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 prior to 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 feed ingredients is presented in Table 1.

2.2 Chemical analysis and calculations

The dry matter, crude protein and ether extract content of feed and faecal samples were determined according to standard AOAC (2005) [9] procedures, while neutral detergent fibre, acid detergent fibre and lignin were determined by the method of Van Soest *et al.* (1991) [10]. Total digestible nutrients were calculated according to NRC (2001) guidelines, and nutrient digestibility was estimated using the indirect digestibility method of Wallace *et al.* (1970) [11], as follows:

3.2 Fortnightly body weight change and dry matter intake

Fortnightly changes in body weight are presented in Fig. 1. The initial body weight of Deoni calves was 133.88 kg in the control group and 134.48 kg in the treatment group. Body weight at the first, second and third fortnightly intervals was 137.41, 143.69 and 150.75 kg in the control group and 137.84, 144.66 and 152.73 kg in the treatment group, with a final body weight of 157.74 kg in the control group and 158.96 kg in the treatment group. Body weight increased steadily in both groups over the trial period, and the difference between groups was not significant at any stage ($p > 0.05$).

Fortnightly dry matter intake is presented in Fig. 2. Dry matter intake at the initial, first, second and third fortnightly intervals was 3.80, 3.78, 4.26 and 4.41 kg/d in the control group compared with 3.99, 3.91, 4.10 and 4.63 kg/d in the treatment group, with a mean intake of 4.11 kg/d in the control group and 4.58 kg/d in the treatment group. Dry matter intake did not differ significantly between groups at any fortnightly interval.

3.3 Nutrient intake

Nutrient intake during the digestibility trial is presented in Table 2. Dry matter intake was 4.70 kg/d in the control group and 4.81 kg/d in the treatment group, and organic matter intake was 4.16 and 4.09 kg/d, respectively. Crude protein intake was 462.68 g/d in the control group and 469.83 g/d in the treatment group, while ether extract intake was 155.22 and 154.48 g/d, respectively. Neutral detergent fibre intake was 2.38 and 2.28 kg/d, and acid detergent fibre intake was 1.50 and 1.41 kg/d, in the control and treatment groups, respectively. Mean total digestible nutrient intake was 2.52 kg/d in the control group and 2.55 kg/d in the treatment group. None of these differences between groups was statistically significant ($p > 0.05$).

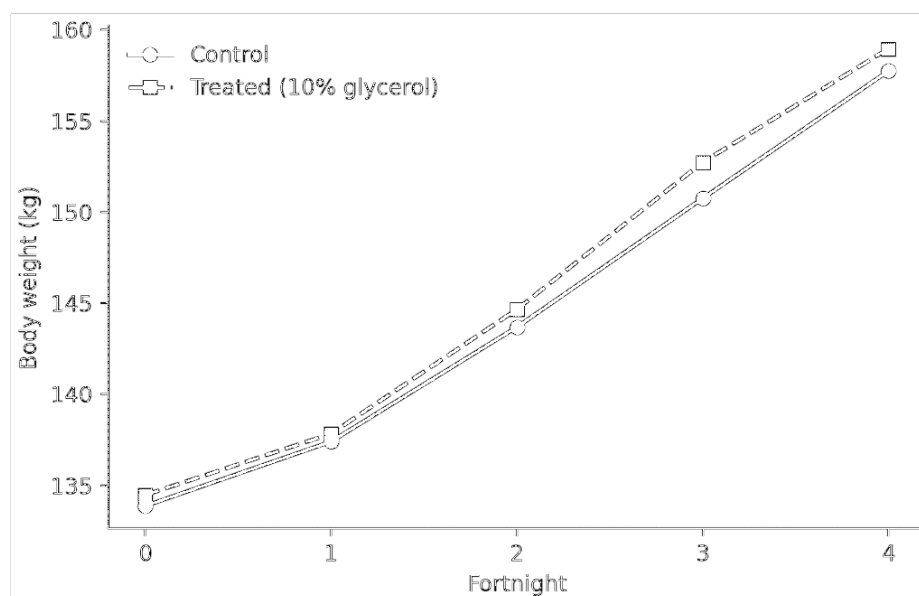


Figure 1. Fortnightly body weight (kg) of Deoni growing calves during the experiment

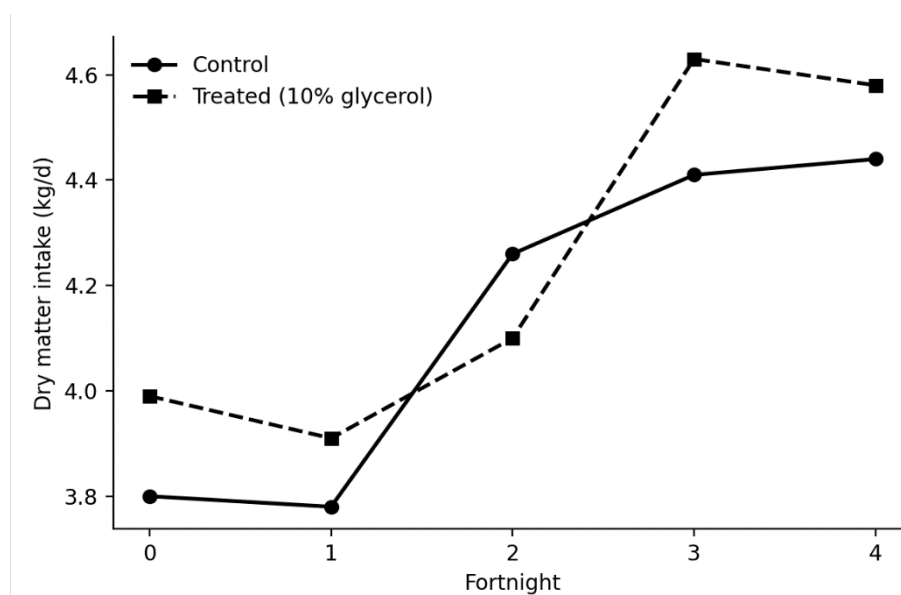


Figure 2. Fortnightly dry matter intake (kg/d) of Deoni calves during the experiment

3.4 Nutrient digestibility and nutritive value

Nutrient digestibility coefficients are presented in Fig. 3. Dry matter digestibility was 61.56 percent in the control group and 60.40 percent in the treatment group, with corresponding organic matter digestibility of 62.78 and 61.79 percent. Crude protein digestibility was 65.49 and 66.66 percent, and neutral detergent fibre and acid detergent fibre digestibility were 50.79 and 38.32 percent in the control group compared with 51.23 and 37.92 percent in the treatment group. None of these differences between groups was significant ($p > 0.05$). Crude protein, digestible crude protein and total digestible nutrient content of the diets were 10.88 and 10.92 percent, 7.33 and 7.19 percent, and 60.48 and 59.26 percent for the control and treatment groups, respectively, with no significant difference between groups.

4. Discussion

4.1 Nutrient composition of the experimental diet

Dietary neutral detergent fibre content is an important determinant of voluntary intake and rumen fill in dairy cattle (Paulson, 2009). The basal diet in the present study was formulated at a roughage-to-concentrate ratio of 70:30 (Section 2.1), and the comparatively high neutral detergent fibre content of ragi straw (73.41 percent) would therefore have contributed substantially to the total dietary fibre load and

rumen fill in both dietary groups. Because the same basal diet and roughage-to-concentrate ratio were maintained across treatments, this fibre-related influence on rumen fill would be expected to have acted similarly in the control and glycerol-supplemented groups, consistent with the comparable dry matter intake recorded between them (Section 3.2).

4.2 Body weight change and dry matter intake

These findings are consistent with reports that inclusion of glycerol in the concentrate had no effect on body weight gain or feed efficiency in growing cattle, in contrast to reports of reduced average daily gain in lambs fed high levels of glycerol, attributed to reduced dry matter intake associated with the high fatty acid concentration of some glycerol sources (Almeida *et al.*, 2017) [1].

This is comparable to earlier findings in Korean cattle steers, in which glycerol supplementation increased concentrate intake without a significant effect on overall dry matter intake, whereas dry matter intake declined progressively as dietary glycerol level increased from 0 to 240 g/kg in crossbred calves (Maciel *et al.*, 2020) [12].

4.3 Nutrient intake

A reduction in dry matter intake associated with glycerol feeding has been attributed to hepatic oxidation of the propionate produced from ruminal glycerol fermentation (Allen *et al.*, 2013) [13], and dry matter

intake has been reported to decline progressively as dietary glycerol level increases from 0 to 240 g/kg in crossbred calves (Maciel *et al.*, 2020) [12]. The effect of crude glycerol supplementation on intake appears to depend on its purity, dose and the category of animal, as well

as on the composition of the basal diet; a possible association between reduced crude protein, neutral detergent fibre and non-fibrous carbohydrate intake and reduced dry matter intake at higher glycerol inclusion levels has also been reported (Maciel *et al.*, 2020) [12].

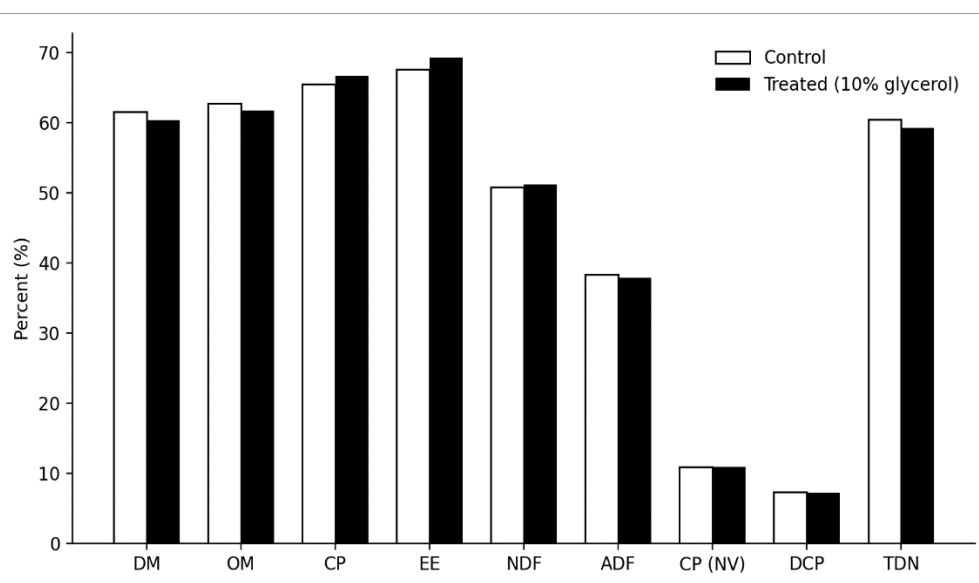


Figure 3. Effect of glycerol supplementation on nutrient digestibility and nutritive value of the diet in Deoni calves

4.4 Nutrient digestibility and nutritive value

These results are consistent with reports of no significant effect of glycerol inclusion on nutrient digestibility in lambs (Avila-Stagno *et al.*, 2013) [14] and of no difference in organic matter digestibility when glycerol replaced alfalfa in cattle diets (Rémond *et al.*, 1993) [15]. In contrast, dry matter digestibility has been reported to increase when glycerol was supplemented at 0 to 3 percent of dietary dry matter (Wang *et al.*, 2009) [16], and in vitro digestibility has similarly been reported to increase with glycerol inclusion in concentrate diets.

4.5 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 feeding and digestibility trial, it is modest relative to larger confirmatory studies, and international reviewers may reason-

ably scrutinize it. The consistent trends observed here across body weight, intake and digestibility measures nonetheless support this work as a valuable preliminary study, and larger-scale trials are recommended to confirm these findings before wider application in indigenous cattle production systems.

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.

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

Table 2. Effect of glycerol supplementation on nutrient intake in Deoni calves

Parameter	Control	Treated group	SEM	P-value
BW (kg)	159.32	160.90	4.33	0.87
DMI (kg/d)	4.70	4.81	0.17	0.77
DMI (kg/100 kg BW)	2.95	2.98	0.04	0.77
OMI (kg/d)	4.16	4.09	0.14	0.82

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OMI (kg/100 kg BW)	2.61	2.54	0.04	0.33
CP intake (g/d)	462.68	469.83	14.24	0.82
CP intake (g/100 kg BW)	290.43	291.60	1.79	0.76
EE intake (g/d)	155.22	154.48	3.94	0.93
EE intake (g/100 kg BW)	97.40	96.15	0.65	0.37
NDF intake (kg/d)	2.38	2.28	0.08	0.54
NDF intake (kg/100 kg BW)	1.50	1.41	0.03	0.12
ADF intake (kg/d)	1.31	1.30	0.05	0.93
ADF intake (kg/100 kg BW)	0.82	0.81	0.01	0.46
TDN intake (kg/d)	2.52	2.55	0.07	0.85
TDN intake (kg/100 kg BW)	1.62	1.58	0.05	0.78

5. Conclusion

Under the conditions of this study, dietary inclusion of crude glycerol at 10 percent of the concentrate mixture did not adversely affect feed intake, growth performance or nutrient digestibility of Deoni calves. These findings suggest that crude glycerol can be safely incorporated into the concentrate of growing indigenous calves as an alternative, non-conventional energy source, supporting its use in sustainable ruminant feeding systems. 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.

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