

## Journal of the Hellenic Veterinary Medical Society

Vol 71, No 4 (2020)



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doi: [10.12681/jhvms.25917](https://doi.org/10.12681/jhvms.25917)

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### To cite this article:

AHMED, S., AAMIR, M., UL-HAQUE, M. N., AHMAD, N., MARGHAZANI, I., & KHAN, M. I. (2021). Influence of rumen bypass fat fed with total mixed ration on growth performance in Nili-Ravi buffalo calves. *Journal of the Hellenic Veterinary Medical Society*, 71(4), 2437–2444. <https://doi.org/10.12681/jhvms.25917>

## **Influence of rumen bypass fat fed with total mixed ration on growth performance in Nili-Ravi buffalo calves**

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**ABSTRACT:** This study was conducted to know the influence of rumen bypass fat supplement on growth performance of Nili-Ravi mal buffalo calves. Nili Ravi buffalo male calves (n=12) were randomly selected and divided into two groups i.e., A and B based on two different levels of age. These groups (A and B) were further divided into two respective subgroups i.e., A1, A2 and B1, B2. Sub groups A1 and B1 served as control (without supplement) whilst subgroups A2 and B2 were fed RBF supplement (at the rate of 2.35% of dry matter intake) with basal diet with total mixed ration. Results showed no statistical difference ( $P>0.05$ ) in dry matter intake, body weight gain and body condition score on rumen bypass fat supplementation. In blood metabolites, rumen bypass fat supplementation increased ( $P<0.05$ ) blood triglyceride and cholesterol levels, however, it reduced ( $P<0.05$ ) blood glucose level in Nili-Ravi buffalo male calves. This study suggests that supplementation of rumen bypass fat at the rate of 2.35% per day in TMR possess no impact on growth performance parameters in Nili Ravi buffalo male calves.

**Keywords:** buffalo calves, rumen bypass fat, growth performance, body condition score, blood metabolites

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*Date of initial submission: 06-04-2019*  
*Date of revised submission: 09-06-2020*  
*Date of acceptance: 17-06-2020*

## INTRODUCTION

Nutrition plays key role in the maintenance and body development of animals (İmran *et al.*, 2018; Nawab *et al.*, 2019; Aydogdu *et al.*, 2019). The available information on the nutrients requirements of buffalo male calves for growth is limited. Of all nutrients, energy is an important constituent of a ration for beef animals (Basra *et al.*, 2003). Traditionally, cereal grains are energy sources which are used for feeding of ruminants, possessing good production potential however, high levels of grains adversely affect feed intake, fermentation characteristics of rumen and consequently digestion (Palmquist and Jenkins, 1982). The growth potential of calves can be fully exploited by incorporating fats in the ration. Rumen bypass fat is vegetable oil which contains 85% palmitic acid and has higher melting point, it bypasses rumen degradation and does not melt in rumen. This undegraded bypass fat degraded by lipase enzyme in small intestine (Singh *et al.*, 2014). Rumen bypass fat remain partly unaffected by process of bio-hydrogenation of rumen microorganisms and hence it supports in lessening the looming danger of metabolic diseases (Naik *et al.*, 2009). Zinn and Plascencia (1996) stated that consumption of energy by feedlot steers augmented linearly with cumulative nutritional energy density. Moreover, a direct reduction in feed consumption and unfilled body gain is reported due to addition of fat in the diets of steers (Zinn, 1989; Jenkins and Jenny, 1989). In dissimilarity, Palmquist and Jenkins (1982) stated that calcium could get better fiber digestibility in additional fat diets by creating unsolvable soaps, which eliminate fatty acids (FA) from rumen fluid, therefore they are not available for a long time for rumen bacteria. Though, Sutton *et al.* (1983) stated that protected linseed oil caused a large depression in neutral detergent fiber breakdown in the rumen. It was designated that the seeming discrepancy might be due to the extent of defence, and the result fatty acid slowly released in the rumen which permissible hydrogenation of fatty acid to happen, therefore decreased microbial activity. Considering varying nature of reported literature due to different species, physiological status of experimental animals, the protection level of rumen bypass fat (RBF), basal diet and many other reasons, this study was designed to know the influence of RBF supplemented with total mixed ration (TMR) on growth performance in Nili-Ravi buffalo male calves.

## MATERIALS AND METHODS

This study was carried out in University of Veterinary and Animal Sciences, Ravi campus, Pakistan. For this purpose, Nili Ravi buffalo male calves (n=12) were randomly selected and divided into two groups i.e., A and B based on age. The age of buffalo calves in group A were 21-24 months (average body weight  $308 \pm 8.8$ kg) whilst age of buffalo calves in group B were 08-11 months (average body weight  $130 \pm 7.6$ kg). These groups (A and B) were further divided into two respective subgroups i.e., A1, A2 and B1, B2. Subgroups A1 and B1 served as control (without supplement whilst subgroups A2 and B2 were fed RBF supplement (@ 2.35% of dry matter intake). RBF (in fine flanks, white to pale in color) is commercially available product with different trade names and are prepared by 100% vegetable oil. This product was made by palm oil fatty acids and fractionated by physical method (where in fatty acids of wanted configuration were achieved). It contained 99 % total fat, 85% palmitic acid, 20% stearic acid with 29 ( MJ/kg) NEL and 56-58°C melting point. This product is developed to provide maximum energy from a protected fat with great small intestine digestibility and low rumen solubility.

As basal diet, total mixed ration (Table 1) containing 2.5 Mcal/kg metabolizable energy and 12% crude protein fed to all groups at the rate of 3% body weight on dry matter basis. The duration of experiment lasted for 70 days with 10 days adjustment period of calves to their respective diets. Clean and freshwater was given *ad libitum* daily for 24 hours. The refusals were collected and recorded (if any). Nili Ravi Buffalo calves were de-wormed for ecto and endo-parasites during the adjustment period.

Data on daily feed intake was recorded on daily basis. Body weight of individual Nili-Ravi buffalo were taken before the commencement of feeding experiment, subsequent weight of each Nili Ravi calf was recorded after every 14 days using a digital weighing balance. Body weight gain (kg) of each calf was determined by deducting initial body weight from final body weight. Body condition score (BCS) was recorded by using 1 to 9 measurement scale in which different scores were allotted according to the body conditions of animals with 1 being very thin and 9 being extremely obese (Eversole *et al.*, 2009). BCS in terms of brisket, ribs, pins, hooks, back and tail head of every male calf was noted before the start of experiment and at the end of feeding trial.

**Table 1.** Ingredient and chemical composition of TMR fed to experimental calves

| Ingredients %      | Control group | Supplemented group |
|--------------------|---------------|--------------------|
| Maize grains       | 25.00         | 20.00              |
| Soybean meal       | 05.00         | 05.00              |
| Canola meal        | 06.00         | 06.00              |
| Molasses           | 06.00         | 06.00              |
| Oat silage         | 45.00         | 47.65              |
| Wheat straw        | 11.00         | 11.00              |
| Mineral mixture    | 02.00         | 02.00              |
| RBF                | 0.00          | 2.35               |
| Total              | 100           | 100                |
| <b>Nutrients %</b> |               |                    |
| DM                 | 56.90         | 56.90              |
| CP                 | 12.00         | 12.00              |
| ME (Mcal/kg)       | 2.50          | 2.50               |
| NDF                | 28.70         | 28.70              |
| NFC                | 38.34         | 38.34              |
| Calcium            | 0.98          | 0.98               |
| Phosphorus         | 0.40          | 0.40               |
| Fat                | 2.08          | 4.07               |

TMR Total mixed ration; RBF= Rumen bypass fat; DM= Dry matter CP= Crude protein;

M.E= Metabolizable energy; NDF= Neutral detergent fiber;

NFC= Non fiber carbohydrates

Blood samples of Nili-Ravi buffalo male calves were taken at day zero of experiment and then at the completion of experiment by puncturing the jugular vein in 20 ml sterilized disposable syringes and were transferred to EDTA coated tubes for biochemistry analysis. Plasma was collected from the samples of blood by the process of centrifugation at 3000 rpm for five minutes in a centrifuge. All samples were labeled carefully and plasma was stored at the temperature of -20 °C until further analysis (Ashmawy, 2015). The blood glucose, cholesterol and triglyceride levels were measured by standard procedure using Human cat # 10260, 10017 and 12851, respectively.

Collected data on all studied parameters were statistically analysed (Steel *et al.*, 1997) under 2x2 factorial design using Statistical Analysis System software (SAS, 2004).

## RESULTS AND DISCUSSION

Daily average and total dry matter intake (Mean  $\pm$  S.E) of Nili-Ravi buffalo male calves on experimental diets are given in Table 2. In our findings, feeding additional RBF did not improve average daily matter intake (DMI) and total dry matter intake. Similar response with and without RBF supplementation in DMI may be due to nature of basal diet fulfilling ba-

sic nutrient requirement of calves. In our study, TMR was fed as basal diet and it is well established fact that TMR provides all ingredients containing different nutrients in one bolus. Therefore, it is deduced that it may have attributed to the non-interference of RBF with digestibility of nutrients and its relatively stable nature and minimum dissociation in the rumen (Reddy *et al.*, 2003). Findings of this study are in agreement with the earlier findings (Garg *et al.*, 1997) where feed intake was not different on RBF feeding at the rate of 500 gm per day per head in Holstein Friesian cows. Similarly, Raval *et al.* (2017) reported no difference in DMI on RBF feeding in Surti buffaloes. Tyagi *et al.* (2010) also reported statistically same DMI on RBF feeding at the rate of 2.5% in multiparous crossbred cows. However, some studies have shown increased DM intake on RBF feeding. Gajera *et al.* (2013) performed an experiment on growing Jaffrabadi heifers and reported increased ( $P < 0.05$ ) DMI. Likewise, Sirohi (2010) reported higher DMI with additional bypass fat supplementation in crossbred cows. The increased DMI in these studies may be due to different physiological stage of animals and nature of basal diet.

**Table 2.** Daily DMI\* and total DMI (Mean  $\pm$  SE) intake in Nili-Ravi male buffalo calves fed experimental diets

| Diet                | Average DMI*<br>(kg /animal/) | Total DMI<br>(kg)               |
|---------------------|-------------------------------|---------------------------------|
| Control             | 6.80 $\pm$ 0.90               | 407.86 $\pm$ 54.10              |
| RBF**               | 6.80 $\pm$ 1.06               | 407.82 $\pm$ 63.74              |
| <b>Age (months)</b> |                               |                                 |
| 21-24               | 8.88 $\pm$ 0.20 <sup>a</sup>  | 532.90 $\pm$ 12.09 <sup>a</sup> |
| 8-11                | 4.71 $\pm$ 0.40 <sup>b</sup>  | 282.78 $\pm$ 24.23 <sup>b</sup> |
| <b>P-value</b>      |                               |                                 |
| RBF                 | 0.9990                        | 0.9990                          |
| Age                 | <.0001                        | <.0001                          |

<sup>a-b</sup> different superscript in a column shows significant differences;

\*Dry matter intake ;

\*\*RBF Rumen bypass fat

In the present study, as expected, younger age calves (8-11 months age) consumed less (average daily DMI, Total DMI) as compared to older age calves (21-24 months age). These findings explain higher requirement of DMI in comparatively older age and weight calves. These findings are similar with the Kumar *et al.* (2007) who fed RBF to Murrah buffalo calves and found low DM intake in calves of younger age (less than one year) buffalo calves. The lower DMI in younger age calves may be due to the fact that

unsaturated long chain fatty acids reaching the small intestine may reduce the gastrointestinal motility and thus DMI (Reidelberger 1994). However, Meshram *et al.* (2017) conducted study on growing crossbred calves having less than one year of age and compared RBF fed group with non RBF fed group. They found significantly higher dry matter intake with feeding RBF diets.

Influence of RBF supplementation with TMR on body weight gain (Mean  $\pm$  S.E) in Nili-Ravi buffalo male calves is given in Table 3 and Table 4. In our study, RBF supplementation did not show significant body weight gain as compared to calves fed same diet without RBF supplementation. However, age

and weight variation influenced body weight gain. Earlier studies (Wadhwa *et al.*, 2012; Long *et al.*, 2014; Len *et al.*, 2016; Mangrum *et al.*, 2016) are in agreement to our findings where weight gain in experimental animals was increased with supplementation of RBF. However, in his studies Vahora *et al.* (2012) found increased ( $P < 0.05$ ) body weight gain in growing buffalo calves on RBF feeding. Higher gain on RBF supplementation in some studies may be correlated with nature of basal diet and consequent nutrient digestibility, nitrogen balance and utilization, better rumen environment and higher microbial-nitrogen synthesis. (Bhatti *et al.*, 2016).

**Table 3.** Fortnight body weight (Mean  $\pm$  SE) in Nili-Ravi buffalo calves fed experimental diets

| Diet                | 0 day<br>(Kg)                   | 14 day<br>(Kg)                  | 28 day<br>(Kg)                  | 42 day<br>(Kg)                  | 60 day<br>(Kg)                  |
|---------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Control             | 255.67 $\pm$ 42.77              | 261.17 $\pm$ 43.06              | 268.00 $\pm$ 43.67              | 279.33 $\pm$ 45.13              | 295.00 $\pm$ 45.77              |
| RBF*                | 266.33 $\pm$ 40.00              | 277.33 $\pm$ 42.95              | 286.83 $\pm$ 43.75              | 298.67 $\pm$ 45.10              | 311.67 $\pm$ 46.21              |
| <b>Age (months)</b> |                                 |                                 |                                 |                                 |                                 |
| 8-11                | 173.50 $\pm$ 13.08 <sup>b</sup> | 177.67 $\pm$ 13.13 <sup>b</sup> | 184.00 $\pm$ 13.37 <sup>b</sup> | 192.67 $\pm$ 13.99 <sup>b</sup> | 205.50 $\pm$ 14.31 <sup>b</sup> |
| 21-24               | 348.50 $\pm$ 14.40 <sup>a</sup> | 360.83 $\pm$ 14.06 <sup>a</sup> | 370.83 $\pm$ 13.66 <sup>a</sup> | 385.33 $\pm$ 14.16 <sup>a</sup> | 401.17 $\pm$ 14.99 <sup>a</sup> |
| <b>P-value</b>      |                                 |                                 |                                 |                                 |                                 |
| RBF                 | 0.8591                          | 0.7958                          | 0.7668                          | 0.7681                          | 0.8030                          |
| Age                 | <.0001                          | <.0001                          | <.0001                          | <.0001                          | <.0001                          |

<sup>a-b</sup> different superscript in a column shows significant differences; \*RBF= Rumen bypass fat

**Table 4.** Weight gain (Mean  $\pm$  SE) in Nili-Ravi buffalo calves fed experimental diets

| Diet                | 14 day<br>(Kg)                | 28 day<br>(Kg)   | 42 day<br>(Kg)                | 60 day<br>(Kg)   | ADG/anim<br>(kg)             | AGF<br>(Kg)                   | TWG<br>(Kg)                   |
|---------------------|-------------------------------|------------------|-------------------------------|------------------|------------------------------|-------------------------------|-------------------------------|
| Control             | 5.50 $\pm$ 1.23               | 6.83 $\pm$ 1.74  | 11.33 $\pm$ 1.58              | 15.67 $\pm$ 1.23 | 0.66 $\pm$ 0.07              | 9.83 $\pm$ 1.05               | 39.33 $\pm$ 4.22              |
| RBF                 | 11.00 $\pm$ 3.46              | 9.50 $\pm$ 2.39  | 11.83 $\pm$ 1.74              | 13.00 $\pm$ 1.21 | 0.76 $\pm$ 0.12              | 11.33 $\pm$ 1.73              | 45.33 $\pm$ 6.92              |
| <b>Age (months)</b> |                               |                  |                               |                  |                              |                               |                               |
| 8-11                | 4.17 $\pm$ 0.79 <sup>b</sup>  | 6.33 $\pm$ 2.01  | 8.67 $\pm$ 1.09 <sup>b</sup>  | 12.83 $\pm$ 1.30 | 0.53 $\pm$ 0.05 <sup>b</sup> | 8.00 $\pm$ 0.76 <sup>b</sup>  | 32.00 $\pm$ 3.03 <sup>b</sup> |
| 21-24               | 12.33 $\pm$ 3.04 <sup>a</sup> | 10.00 $\pm$ 2.02 | 14.50 $\pm$ 0.99 <sup>a</sup> | 15.83 $\pm$ 1.05 | 0.88 $\pm$ 0.07 <sup>a</sup> | 13.17 $\pm$ 1.04 <sup>a</sup> | 52.67 $\pm$ 4.17 <sup>a</sup> |
| <b>P-value</b>      |                               |                  |                               |                  |                              |                               |                               |
| RBF                 | 0.0427                        | 0.4048           | 0.7673                        | 0.1133           | 0.4761                       | 0.2575                        | 0.2575                        |
| Age                 | 0.0073                        | 0.2611           | 0.0073                        | 0.0805           | 0.0025                       | 0.0030                        | 0.0030                        |

<sup>a-b</sup> different superscript in a column shows significant differences

ADG = Average daily gain; AGF = Average weight gain on fortnight basis; TWG = Total weight gain



In our study, average daily and total weight gain increased ( $P<0.05$ ) in older age calves (21-24 months) as compared to younger age (8-11 months) calves which may be due to more weight and age of calves that consequently resulted in increased DMI. In other words, average daily gain was not significantly increased in lower age and weight calves due to less and decreased feed intake (Crystal 2015). Similar to our findings, Gajera *et al.* (2013) reported significant differences ( $P<0.05$ ) with RBF supplementation on body weight gain in Jaffrabadi buffalo heifers in more than one year age).

Initial and final BCS in terms of brisket, ribs, pins, hooks, back and tail head of Nili-Ravi buffalo calves is given Table 5. RBF supplementation and age difference showed no influence on BCS of buffalo calves. These results explain that iso-caloric and iso-nitrogenous nature of basal diet (TMR) may have fulfilled nutritional requirement of calves according to their respective age and weight, hence additional supplementation of RBF may have not further improved BCS. Findings of this study are in accordance with Raval *et al.* (2017) who did not report any change in BCS of Surti buffaloes fed RBF. Likewise, Long *et al.* (2014) reported no difference in BCS of beef heifers fed with different dietary treatments of RBF. On the other hand, an improved BCS in animals with RBF supplementation is reported in literature. Naik *et al.* (2009) recorded significant differences ( $P<0.05$ ) in BCS of cows fed RBF as compared to control diet. However, lactating animals may give repose to RBF supplementation due to high demand of rich energy source in small intestine particularly at early stage of lactation.

**Table 5.** Body condition score (Mean  $\pm$ SE ) in Nili Ravi male buffalo calves fed experimental diets

| Diet                | Day zero (BCS)* | Day Final (Day 60) (BCS) |
|---------------------|-----------------|--------------------------|
| Cnotrol             | 3.00 $\pm$ 0.26 | 4.33 $\pm$ 0.42          |
| RBF**               | 3.00 $\pm$ 0.26 | 5.00 $\pm$ 0.26          |
| <b>Age (months)</b> |                 |                          |
| 8-11                | 2.67 $\pm$ 0.21 | 4.33 $\pm$ 0.33          |
| 21-24               | 3.33 $\pm$ 0.21 | 5.00 $\pm$ 0.37          |
| <b>P-value</b>      |                 |                          |
| RBF                 | 1.0000          | 0.2191                   |
| Age                 | 0.0805          | 0.2191                   |

\*BCS=Body condition score; 1 emaciated; 2 very thin; 3 thin; 4 moderate thin; 5 moderate ideal; 6 moderate fleshy; 7 fleshy; 8 very fleshy; 9 obese.

\*\* RBF= Bypass fat

Effect of diet and age on blood glucose, cholesterol and triglycerides level (Mean $\pm$ S.E, mg/dl) of Nili-Ravi buffalo male calves are given in Table 6. RBF feeding to buffalo calves significantly reduced blood glucose level whilst blood cholesterol and triglycerides level were increased. Findings of this study are in line with Hammon *et al.* (2008) who found significantly reduced glucose concentration in RBF fed animals as compared to control group. The decrease in plasma glucose concentration was not associated with decreased post absorptive availability of glucose, because rate of appearance of glucose was not affected by the diet. Therefore, the reduced glucose concentration was likely due to an increased utilization of glucose (Hammon, 2008). On a similar pattern, in our study the reduced glucose concentration was likely due to increased utilization of glucose. This may be due to elevated fat oxidation in the liver, changes in gastrointestinal hormones, or impact on rumen digestion (Benson *et al.*, 2001). Barley and Baghel (2009) fed RBF at the rate of 100gm per day/buffalo and found significantly increased serum triglyceride levels in buffaloes fed RBF as compared to control group. Our findings are also similar with Ranjan *et al.* (2012) who found significantly increased serum cholesterol level with feeding RBF in Murrah buffaloes. Raval *et al.* (2017) also found that supplementation of RBF significantly ( $P<0.01$ ) increased serum cholesterol and triglyceride levels in Surti buffaloes calves of more than one year age. Increased serum triglycerides and cholesterol levels with the advance in feeding of supplemental RBF might be due to enhanced uptake of dietary fatty acid (Grewal *et al.* 2014). However, contradictory finding were also reported by Tyagi *et al.* (2010) who fed ration 2.5% RBF supplement (on DMI basis) and did not found any effect on cholesterol concentration between groups in cows. In this case, the probable reason might be level of RBF feeding (2.5% of DMI) which was not sufficient to cause increase in serum cholesterol level.

**Table 6.** Blood level (Mean  $\pm$ SE, mg/dl) in Nili-Ravi male buffalo calves fed experimental diets

| Diet                | Glucose          |                                 | Cholesterol        |                                 | Triglyceride       |                                 |
|---------------------|------------------|---------------------------------|--------------------|---------------------------------|--------------------|---------------------------------|
|                     | Initial          | Final                           | Initial            | Final                           | Initial            | Final                           |
| Control             | 99.86 $\pm$ 8.01 | 138.42 $\pm$ 10.86 <sup>a</sup> | 214.34 $\pm$ 10.95 | 228.25 $\pm$ 15.36 <sup>b</sup> | 231.94 $\pm$ 9.13  | 264.62 $\pm$ 10.19 <sup>b</sup> |
| RBF*                | 92.01 $\pm$ 3.52 | 109.51 $\pm$ 5.52 <sup>b</sup>  | 221.09 $\pm$ 15.94 | 365.07 $\pm$ 53.46 <sup>a</sup> | 205.60 $\pm$ 6.18  | 351.76 $\pm$ 44.71 <sup>a</sup> |
| <b>Age (months)</b> |                  |                                 |                    |                                 |                    |                                 |
| 8-11                | 95.86 $\pm$ 6.36 | 113.65 $\pm$ 9.48               | 227.89 $\pm$ 14.45 | 251.74 $\pm$ 20.38 <sup>b</sup> | 214.97 $\pm$ 10.17 | 262.85 $\pm$ 11.00 <sup>b</sup> |
| 21-24               | 96.00 $\pm$ 6.49 | 134.29 $\pm$ 9.97               | 207.54 $\pm$ 11.33 | 341.58 $\pm$ 61.19 <sup>a</sup> | 222.57 $\pm$ 9.05  | 353.53 $\pm$ 43.81 <sup>a</sup> |
| <b>P-value</b>      |                  |                                 |                    |                                 |                    |                                 |
| RBF                 | 0.4450           | 0.0361                          | 0.7371             | 0.0072                          | 0.0578             | 0.0074                          |
| Age                 | 0.9891           | 0.1102                          | 0.3248             | 0.0467                          | 0.5413             | 0.0060                          |

<sup>a-b</sup> different superscript in a column shows significant differences; \*RBF=Rumen bypass fat

Influence of different age groups on blood metabolites in calves, Kumar and Thakur (2007) also reported no influence on blood glucose level in different age groups of Murrah buffalo calves. The research may be due to more utilization of glucose (high metabolic rate) and other factors (homeostatic mechanism) in ruminants and other animals that limits glucose level (Tyagi and Thakur, 2007). Further, similar to our findings, Sharma *et al.* (2016) reported significantly increased cholesterol level in more than one year old buffalo calves. The higher plasma cholesterol in buffaloes may be due to the positive energy balance of animals associated with prilled fat feeding (Ranjan *et al.*, 2012).

## CONCLUSION

It is concluded that supplementation of RBF at the rate of 2.35% per kg in total mixed ration did not improve dry matter intake, body weight gain and body condition score. In blood metabolites, RBF supplementation significantly reduced blood glucose level however, it increased blood triglyceride and cholesterol levels in Nili-Ravi buffalo male calves.

## CONFLICT OF INTEREST

In this research trial, the authors declare that there is no conflict of interests regarding the publication of this article

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