International Journal of Bio-resource and Stress Management https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM November 2024, 15(11): 01-05 **Article** AR5653

Research Article *Stress Management*

DOI: HTTPS://DOI.ORG/10.23910/1.2024.5653

Ground Flaxseed Supplementation Improved the Energy Utilization and Reduced Oxidative Stress in Buffalo Calves

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ABSTRACT

The present study was conducted at the Department of Veterinary Physiology & Biochemistry and Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India during the period of October, 2018 to November, 2019 to evaluate the effect of ground flaxseed supplementation on blood biochemical and metabolic status of buffalo calves. The experiment was performed on four apparently healthy male buffalo calves of 2.0–2.5 years age in this present investigation. They were divided in 2 groups in a switch over design. The animals of Group I were kept as control. The animals of group II were supplemented with Ground Flaxseed @ 15% on dry matter basis replacing oil seed cakes in TMR for 21 days. Blood samples were collected at weekly intervals over a period of 21 days. The results revealed a significant (p<0.05) decrease in plasma glucose and cholesterol values in group II supplemented with ground flaxseed. There was no significant alteration with respect to the plasma total protein, triglycerides, creatinine, urea and total immunoglobulins in any of the groups. The erythrocytic lipid peroxidation was significantly reduced in the flaxseed supplemented group as compared to non-supplemented group. Thus, the present investigation revealed that flaxseed supplementation improved the energy utilization reduced the oxidative stress in animals.

KEYWORDS: Buffalo calves, biochemical parameters, blood, flaxseed oxidative stress

***Citation* (VANCOUVER):** Singh et al., Ground Flaxseed Supplementation Improved the Energy Utilization and Reduced Oxidative Stress in Buffalo Calves. *International Journal of Bio-resource and Stress Management,* 2024; 15(11), 01-05. HTTPS://DOI.ORG/10.23910/1.2024.5653.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 01st August 2024 RECEIVED in revised form on 20th October 2024 ACCEPTED in final form on 08th November 2024 PUBLISHED on 12th November 2024

1. INTRODUCTION

Flaxseed is one of the world's oldest oilseed crops. In the years 2016–2020, the average cultivated area of flaxseed in the world was about 3.39 mha (Cui et al., 2022). Flaxseed is an oilseed which can be used as a source of high-quality protein and fat for ruminants (Neveu et al., 2014). Flaxseed contains high levels of linolenic acid, averaging 18% of the total seed weight and 53% of the total fatty acids (Mustafa et al., 2002). Flaxseed's nutritive value is further enriched by its lipid profile, characterized by the increased unsaturated fatty acid (UFA) content; omega-3 fatty acids (ω-3) account for more than 50% of the total lipids. These fatty acids (FAs) are positively associated with human health by supporting cardiovascular function. (Swanson et al., 2012). Concurrently, ω-3 were investigated as cow fertility enhancers and proven to support estrus length and intensity of cow's typical estrus behavior (Zachut et al., 2010). Although rumen microbial metabolic activities cause UFA biohydrogenation, a process which modifies UFA to saturated fatty acids, it was shown that the inclusion of 25 g kg^{-1} DM of flaxseed oil in dairy cow rations resulted in increased ω-3 fatty acid concentrations in milk (Yoshimura et al., 2018) As a source of omega-3 polyunsaturated fatty acid (n-3 PUFA), flaxseed is widely used to enhance levels of n-3 PUFA in milk production (Meignan et al., 2017; Brzozowska et al., 2018; Marino et al., 2019), particularly, α-linolenic acid (ALA; c9,c12,c15-C18:3). Intake of flaxseed or oil by dairy ruminants has also been shown to mitigate methane production (Chilliard et al., 2009). Fat supplements such as linseeds are included in the diets of ruminants to increase energy density, improve nutrient utilization, enhance milk and meat yields, and modify fatty acid (FA) composition (Soder et al., 2013). The main components of protein in FSM are globulin and albumin (Nwachukwu et al., 2018). The concentration and composition of essential amino acids (AAs) in FSM are similar to those in soybeans meals (Shim et al., 2015). In addition, flaxseed proteins can be hydrolyzed by proteases to produce biologically active peptides. Active peptides play important physiological roles in the body with anti-inflammatory and antioxidant properties (Marambe et al., 2008). Flaxseed protein is considered a good source of plant protein. Adding an appropriate amount of FSM to livestock and poultry diets can improve animal immunity, thereby improving animal production performance and related livestock product flavor (Kumar et al., 2019). The application of FSM in ruminants mainly focuses on dairy cows. It is particularly important to provide appropriate feed for dairy cows during the milk production phase since the nutrient content of milk is susceptible to diet. The production of n-3 PUFA-rich milk by supplementing the diet with flaxseed to improve human health has attracted more and more attention in animal production. Plenty of studies have reported that the supplementation of flaxseed in dairy cows' diets is effective in increasing the levels of n-3 PUFA in milk (Petit et al., 2010; Petit et al., 2010; Huang et al., 2022; Huang et al., 2021). Therefore, keeping in view, the importance of flaxseed the present study was planned to evaluate the effect of flaxseed on biochemical and metabolic profile of buffalo calves.

2. MATERIALS AND METHODS

The present study was conducted at the Department of Veterinary Physiology & Biochemistry and Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Experimental protocols using buffalo calves in this study had been recommended by the Institutional Ethical committee (IAEC) of the University and approved by Committee for the Purpose of Control and supervision of experiments on Animals (CPCSEA). Animal Welfare Division, Ministry of Environment, Forest and Climate Change, Government of India. All the experiments with buffalo calves were carried out according to the guidelines of IAEC.

The experiment was performed on four apparently healthy male buffalo calves of 2.0–2.5 years age in the present investigation. All the animals were fed on complete feed. as per Anonymous (2013). The experimental animals were divided into two groups of four each in a switch over design with a washout period as follows.

Group I (Control): Four male buffalo calves. No supplementation was done to these animals.

Group II (Treatment): Four male buffalo calves. Supplemented with ground flaxseed @ 15 percent on dry matter basis replacing oil seed cakes in TMR for 21 days.

Three blood samples (6–8 ml each), were collected from both the groups at weekly intervals over a period of 21 days in heparinized glass vials by jugular veni-puncture. The blood samples were analyzed shortly after collection for hematological parameters viz. hemoglobin and packed cell volume and rest of samples were processed for the separation of plasma and preparation of hemolysate. The upper meniscus of the blood sample in each collection vial was marked and samples were immediately centrifuged at 2500–3000 rpm for 30 minutes. Plasma was separated and stored in small aliquots at -20°C for analysis of various parameters. Buffy coat was removed and sediment (erythrocyte pellet) was washed thrice with normal saline solution. Distilled water was added up to the marked level and the resulting hemolysate was stored at -20°C till analyzed for erythrocytic lipid peroxidation (LPO) as per Placer et al. (1966) and Nishikimi et al. (1972) respectively. Rest of the parameters viz. plasma total protein, glucose (For glucose samples were collected with sodium fluoride as

anticoagulant), cholesterol, triglycerides, urea, creatinine and total immunoglobulins. were estimated with automatic BPC biosed chemistry analyser within one week of collection using BPC biosed kits. The data were analyzed by simple ANOVA, for comparison of means (Snedecor and Cochran, 1994), by using SPSS (2012).

3. RESULTS AND DISCUSSION

The results of the present study are presented in Table 1. The results of plasma glucose concentration in buffalo calves of group I and group II were 67.26±2.002 and 57.26±2.002 mg dl^{-1}, respectively. Similarly, Canfield et al. (1984) evaluated different biochemical parameters in normal mature swamp buffaloes and reported that mean plasma value of glucose, was 61.2±48.6 mg dl^{-1}. Results revealed that the overall mean plasma glucose concentration was significantly decreased following feeding of ground flaxseed. Similarly, Mesgaran et al. (2012) reported that

plasma glucose concentration was found significantly lower in ground flaxseed in comparison to whole flaxseed fed cows. The fall in blood glucose concentration following flaxseed supplementation may be due to improved glucose utilization as an energy source following hypocholesterolemic effect of flaxseed.

The results of mean plasma total protein concentration in buffalo revealed no significant alteration in group II (treatment) as compared to group I (control). However, Marino et al. (2018) observed that lambs supplemented with linseed depicted higher plasma levels of total protein (p<0.05) than Control and Quinoa groups.

The overall mean plasma cholesterol concentration revealed significant reduction in plasma cholesterol concentration in group II as compared to group I. Similarly, Opyd

Table 1: Biochemical parameters in male buffalo calves supplemented with ground flaxseed

Sampling on (Days)	7	14	21	Overall mean± SE
Blood glucose concentration (mg dl^{-1})				
Group I (control)	64.45±2.995	67.63±4.516	69.71±3.509	67.26±2.022A
Group II (treatment)	60.36±2.039	62.68±4.774	48.75±2.218	57.26±2.022B
Plasma total protein (g %)				
Group I (control)	8.13±0.175	8.16±0.340	8.23±0.208	8.17±0.171A
Group II (treatment)	8.21±0.427	8.11±0.268	8.28±0.283	8.20±0.171A
Plasma cholesterol concentration (mg dl^{-1})				
Group I (control)	64.45±2.996	62.51±3.049	66.76±1.358	64.57±1.671A
Group II (treatment)	59.30±2.807	61.35±4.163	48.75±2.218	56.47±1.671B
Creatinine (mg dl^{-1})				
Group I (control)	1.02±0.152	0.93±0.057	0.99±0.122	0.98±0.081A
Group II (treatment)	1.09±0.093	1.28±0.209	1.12±0.151	1.16±0.081A
Urea (mg dl^{-1})				
Group I (control)	33.50±5.475	35.48±6.099	36.42±5.317	35.13±4.068A
Group II (treatment)	36.40±5.938	37.98±11.049	36.60±6.716	36.99±4.068A
Triglyceride (mg dl^{-1})				
Group I (control)	25.18±4.127	24.85±5.566	18.95±3.343	22.99±2.194A
Group II (treatment)	19.68±3.432	20.37±2.422	22.82±3.132	20.96±2.194A
Total immunoglobulin (mg dl^{-1})				
GroupI (control)	0.43±0.034	0.43±0.033	0.42±0.033	0.43±0.018A
Group II (treatment)	0.39±0.000	0.43±0.032	0.50±0.033	0.44±0.018A
Lipid peroxidation (nmol MDA produced/g/Hb)				
Group I (control)	323.85±13.234	316.95±11.637	317.30±22.821	319.37±9.973A
Group II (treatment)	305.40±11.907	267.95±7.722	253.15±27.522	275.50±9.973B

Each value is a mean of 12 observations representing triplicate samples from 4 experimental animals. Overall mean with different superscripts within groups differ significantly (p<0.05)

et al. (2018) observed significantly decreased HDL cholesterol concentration in the High Fat (flaxseed) group compared to the control group in rats. The fall in plasma cholesterol levels in flaxseed supplemented group may be due to hypocholesterolemic effect of flaxseed. In contrary, Delbecchi et al. (2001); Petit et al. (2001, 2002) reported that plasma cholesterol and NEFA concentrations were greater (p<0.01) for cows fed flaxseed than for those fed the control diet.

The overall mean plasma creatinine and urea concentrations in group I (control) and group II (treatment) depicted no significant change following supplementation of flaxseed. Omar (2018) also observed no significant alterations in serum creatinine and BUN levels in flaxseed oil treated rats as compared to control.

The overall mean values of triglyceride were 25.18±2.194 and 20.96±2.194 mg dl^{-1} in group I and II, respectively. Present study revealed no significant alteration in triglyceride level in group II following administration of flaxseed. Similarly, Opyd et al. (2018) noticed that regardless of the form of supplementation, dietary flaxseeds increased bacterial glycolytic activity in the distal intestine and decrease hepatic fat, especially triglyceride, accumulation in Wistar rats.

However, Marino et al. (2018) observed significant increase in triglycerides in lambs supplemented with linseed+quinoa than control group. Stuglin and Prasad (2005) also observed that feeding flaxseed diet in Wistar rats. resulted in no significant alteration in serum total cholesterol, HDL-C, LDL-C, and VLDL-C concentrations, but serum triglycerides levels were elevated.

The mean values erythrocytic LPO in buffalo calves were 319.37±9.973 and 275.50±9.973 nmol MDA produced/g/Hb in group I and II, respectively. The results revealed significant reduction Erythrocytic LPO level in flaxseed supplemented buffalo claves (Group II) as compared to control (Group I). This decrease in lipid peroxidation might be associated with improved energy utilization and hypocholesterolemic effect of flaxseed supplementation. Similarly Opyd et al. (2018) noticed in an experiment lasting 8 weeks conducted on Wistar rats allocated to four groups as a control group fed with a standard diet; a high-fat (HF) group fed with a diet containing 21% fat and 0.1% cholic acid as a stimulator of lipid absorption; an HF group fed a diet supplemented with 1% native flaxseeds; and an HF group fed a diet supplemented with 1% defatted flaxseeds that both flaxseed forms decreased lipid peroxidation in the kidneys as compared to control.

4. CONCLUSION

The ground flaxseed supplementation in buffalo calves improved the energy utilization and reduced the oxidative stress in animals.

5. ACKNOWLEDGEMENT

The authors express their gratitude to the Head, Veterinary Physiology & Biochemistry, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, for providing necessary facilities to carry out research work successfully.

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