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# Effect of n-3 polyunsaturated fatty acid rich fish oil supplementation on serum biochemical profile in goat

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ABSTRACT

The present study was designed to determine the effect of different levels of n-3 PUFA rich fish oil supplementation on serum biochemical profiles in goat. Goats (20) were divided into 4 equal groups (n,5) and supplemented with one of the 4 levels of EPA and DHA enriched fish oil to provide 0 mg (Control; CG); 78 mg/kg BW (T1); 156 mg/kg BW (T2); and 312 mg/kg BW (T3) doses. The diets in all the groups were made isocaloric by adding palm oil. There was a treatment and day interaction for serum cholesterol concentration. Serum cholesterol in fish oil supplemented groups decreased with the increase in dose and indicated potential effect of n-3 PUFA rich fish oil on reducing the circulatory cholesterol concentration. There was a treatment and day interaction for serum triglycerides concentration at probability of 10%. The mean triglycerides concentration was lower in T2 and T3 on day 20 of supplementation than T1. Further, no effect of supplementation could be observed on serum NEFA concentration on different days of sampling. However, serum BHBA concentration had a treatment and day interaction. The mean concentration was lower in T3 than CG on day 38 of supplementation. The finding suggested that n-3 PUFA supplementation influenced serum biochemical profiles and dose-dependent effect was observed on reducing blood cholesterol concentration.

**Key words**: Cholesterol, Fish oil, Goat, n-3 PUFA, Serum biochemical

A positive effect of dietary fat supplementation on reproductive performance in cattle has been reported (Hess *et al.* 2008, Lopes *et al.* 2009). Initially, role of protected fat was considered as an energy supplement during the transition period to improve in reproductive performance. Later (Staples *et al.* 1998) demonstrated that the effect was also due to fatty acids those act as a precursor of progesterone via cholesterol and prostaglandins. Omega-3 polyunsaturated fatty acids (n-3 PUFA) and its ratio to n-6 PUFA may play an important role on animal health, production and reproduction performance (Abayasekara and Wathes, 1999). Flaxseed and forages are good sources of the short-chain n-3 α-linolenic acid (ALA; 18:3 n-3) in ruminant diets, whereas, fish oil and fishmeal are rich in long-chain n-3 eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6 n-3).

There is concern with regard to supplementation of different types of fats in the ration as well as its dose, and particularly types of responses expected through such supplementation in ruminants. The dietary fat supplementation to ruminants affects the fermentation patterns which change nutrients profile absorbed from the gastro-intestinal tract. Long chain PUFA and saturated fats tend to bypass the rumen unaltered and have fewer effects on ruminal fermentation. Hence, it is possible that the chain length of fatty acids and saturation of the fat source may cause considerable variation in serum metabolites (Thomas *et al.* 1997). Circulating cholesterol is the primary precursor for mammalian synthesis of steroid hormones i.e. estradiol and progesterone, hence its concentration may affect the reproduction (Staples *et al.* 1998). In addition, triglycerides, non-esterified fatty acid (NEFA) and beta hydroxyl butyric acid (BHBA) also have a significant effect on the general health and body condition of the animals. A very few studies have been conducted so far on the effect of fat supplementation on blood biochemicals in goats (Teama and El-Tarabany 2016, Adeyemi *et al.* 2016) which remains inconclusive. Therefore, objective of present study was to determine the effect of different levels of n-3 PUFA rich fish oil supplementation on serum biochemical profile with respect to serum cholesterol, triglycerides, NEFA and BHBA in goat.

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## MATERIALS AND METHODS

*Experimental animals and diet:* Goats (20) of Rohilkhand region, aged between 2–2.5 years with 1 to 2

parity were used for this study. All these experimental goats were divided into 4 equal groups (n=5) and were supplemented 1 of 4 levels of a partially rumen protected EPA and DHA enriched fish oil to provide (i) 0 mg (control); (ii) 78 mg/kg BW (low level; T1); (iii) 156 mg/kg BW (Medium level; T2); and (iv) 312 mg/kg BW (higher level; T3). Fish oil was supplemented at 0, 0.3, 0.6 and 1.2 ml/kg BW in control (CG) and treatment groups, respectively. All goats were fed fresh maize fodder (*Zea mays*) @ 0.3 kg/goat/day and *ad lib.* wheat straw. The diets in all the groups were made isonitrogenous and isocaloric by adding palm oil so that the total oil was supplemented to each goat @1.2 ml/kg BW constituting approximately 3.5% (v/w) of total dry matter (DM). All goats were supplemented oil individually after thorough mixing in the concentrate supplement (CS) daily between 1100–1200 h. Each goat was offered 0.25 kg concentrate on DM basis daily to meet the maintenance requirement. Feeding trial consisted of 7 d adjustment period followed by 56 d of experimental period on respective diets of CG and TGs. The ingredient composition and chemical analysis of the CS, maize green and wheat straw are presented in Table 1. The fatty acid composition of the fish and palm oil supplemented are presented in Table 2.

*Blood sampling and biochemical assays:* Blood samples were collected on day –7, 20, 38 and 55 of the 56 d experimental period for measurement of serum cholesterol, triglycerides, NEFA and BHBA. Blood was collected by jugular venipuncture in vacutainers and centrifuged at 1,500 ×g at for 15 min. Serum was harvested and stored at –20°C until analysed. Serum cholesterol and triglycerides concentrations were estimated by CHOD-PAP and GPO-PAP methods, respectively using biochemical kits. In addition, serum NEFA and BHBA concentrations were estimated using ELISA kits (M/s Qayee-Bio, Shanghai Qayee Biotechnology Co., Ltd., Shanghai, China).

Table 1. Ingredient compositions and chemical analysis of the concentrate and forage

| | Concentrate | Green | Straw |
|---|---|---|---|
| Ingredients (g $kg^{-1}$) | | | |
| Wheat bran | 520 | - | - |
| Maize | 250 | - | - |
| Deoiled soya bean meal | 200 | - | - |
| Common salt | 10 | - | - |
| *Mineral mixture | 20 | - | - |
| Chemical composition (g $kg^{-1}$) | | | |
| DM | 958 | 202 | 901.3 |
| CP | 197.2 | 69.1 | 33.9 |
| ADF | 128.1 | 430.1 | 509.9 |
| NDF | 459.6 | 735.4 | 762.2 |
| EE | 31.2 | 40.4 | 10.4 |
| Ash | 73.4 | 84.9 | 97.9 |
| GE (Mcal $kg^{-1}$) | 3.82 | 3.47 | 3.9 |

DM, dry matter; CP, crude protein; ADF, acid detergent fibre; NDF, neutral detergent fibre; EE, ether extract; GE, gross energy.

*Mineral mixture contained per kg of supplement: 220 g Ca, 120g P, 60 g Mg, 1000 mg Cu, 1200 mg Mn, 8000 mg Zn, 4000 mg Fe, 120 mg Co, 260 mg I and 700 mg F

Table 2. Fatty acid composition (g/100 g FA) of fish oil and palm oil supplemented to goats

| Fatty acid | Fish oil | Palm oil |
|---|---|---|
| Lauric acid (C12:0) | 0.22 | 0.35 |
| Myristic acid (C14:0) | 4.69 | 1.37 |
| Myristoleic acid (C14:1) | 0.24 | 0 |
| Pentadecylic acid (C15:0) | 0.80 | 0.08 |
| Palmitic acid (C16:0) | 25.26 | 39.22 |
| Palmitoleic acid (C16:1) | 12.49 | 0.53 |
| Heptadecanoic acid (C17:0) | 0.75 | 0.12 |
| Cis-10-Heptadecanoic acid (C17:1) | 1.63 | 0 |
| Stearic acid (C18:0) | 5.38 | 4.5 |
| Elaidic acid (C18:1 trans-n-9) | 2.75 | 0 |
| Oleic acid (C18:1 cis-n-9) | 9.43 | 39.92 |
| Linoleic acid (C18:3 n-6) | 1.93 | 11.22 |
| Gamma- linolenic acid (C18:3 n-6) | 0.50 | 0 |
| Eicosanoic acid (C20:0) | 0.36 | 0.2 |
| á-Linolenic acid (C18:3 n-3) | 0.20 | 0 |
| Eicosadienoic acid (C20:2 n-6) | 0.14 | 0 |
| Docosanoic acid (C22:0) | 0.19 | 0 |
| Eicosatrienoic acid (C20:3 n-3) | 0.32 | 0 |
| Tricosanoic acid (C23:0) | 2.62 | 0 |
| Docosadienoic acid (C22:2 n-6) | 0.16 | 0 |
| Lignoceric acid (C24:0) | 0.24 | 0.15 |
| Eicosapentaenoic acid (C20:5 n-3) | 16.21 | 0 |
| Nervonic acid (C24:1 n-9) | 0.49 | 0 |
| Docosahexaenoic acid (C22:6 n-3) | 10.15 | 0 |

*Statistical analysis:* Data were tested initially for normal distribution by Shapiro-Wilk test. The data for different serum biochemical variables were analyzed using General linear model repeated measure ANOVA with terms for group, time period and their interactions included in the model and animal within diet set as the error term as follows:

$$Y_{hij} = \mu + \gamma_h + \tau_j + (\gamma\tau)_{hj} + \pi_{i(h)} + e_{hij}$$

$\mu$,grand mean; $\gamma_h$,effect of group h; $\tau_j$,effect of time j; $(\gamma\tau)_{hj}$, interaction of time j by group h; $\pi_{i(h)}$,individual difference component for subject i in group h; and $e_{hij}$, error for subject i in group h at time j.

P values <0.05 were considered as statistically significant. Data analysis was done with SPSS software (IBM® SPSS® statistics, version 20.0).

## RESULTS AND DISCUSSION

The effect of different doses of n-3 PUFA on serum biochemicals i.e. cholesterol, triglycerides, NEFA and BHBA in CG and TGs was investigated on day –7, 20, 38 and 55 of supplementation. The findings indicated an interaction effect between treatment and day for serum cholesterol concentration (P<0.05), however, treatment alone had no significant effect (P>0.05). The mean cholesterol concentration was significantly lower (P<0.05) in T3 than CG on day 55 of supplementation with no significant differences among other TGs (Fig. 1). The

cholesterol concentration in TGs decreased numerically with the increase in the dose of fish oil, thus indicating potential effect of n-3 PUFA rich fish oil on reducing the circulatory cholesterol concentration. In contrast, the cholesterol concentration in CG increased (P<0.05) with palm oil supplementation with progression of time.

Our observations were in conformity with Teama and El-Tarabany (2016) who reported lower serum concentrations of cholesterol in fish oil supplemented goats. It is well established that PUFA of the n-3 family have multiple beneficial effects on chronic heart disease (CHD) risk in human by reducing cholesterol concentration (Illingworth and Schmidt, 1993). There are reports that modulation of cholesterol synthesis is not a major mechanism by which fatty acids affect the plasma cholesterol level; rather, it is likely due to other mechanisms including redistribution of cholesterol between plasma and tissue pools and upregulation of the LDL receptors (Matson and Grundy 1985, Jones *et al.* 1998). In addition, up to 50% of the fatty acids in fish oil have been reported to pass through the rumen unmodified, fish oil is expected to be much less cholesterologenic than either saturated or short chain PUFA rich fats (Byers and Schelling 1988). However, contrast finding were reported by previous workers that n-3 PUFA rich fish meal or fish oil supplementation either increased (Thomas and William 1996, Child *et al.* 2008b) or did not have any effect (Lammoglia *et al.* 1997, Robinson *et al.* 2002, Malik *et al.* 2013) on circulating cholesterol concentrations. In the present study, part feeding of saturated and monounsaturated fatty acid rich palm oil in T1 (3/4 part) and T2 (1/2 part) might be responsible for countering the suppressive effect of long chain n-3 PUFA on serum cholesterol concentration.

There was a treatment and day interaction for serum triglycerides concentration at probability of 10% (P<0.1). There was a significant (P<0.05) effect of treatment on the triglycerides concentration. Its concentration was significantly lower (P<0.05) in T2 and T3 on day 20 of

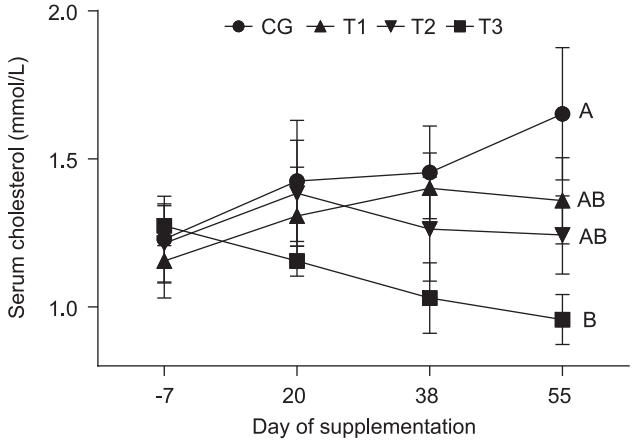


Fig. 1. Serum cholesterol concentrations (mmol/L) in goats fed different levels of n-3 PUFA rich fish oil diets on days –7, 20, 38 and 55 of supplementation. A and B indicate significant (P < 0.05) difference between the groups.

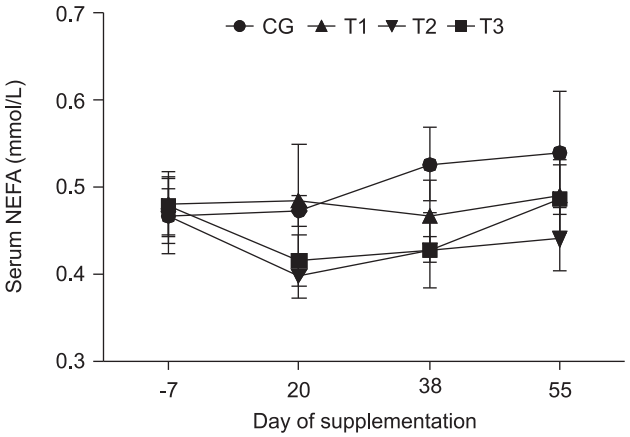


Fig. 3. Serum NEFA concentrations (mmol/L) in goats fed different levels of n-3 PUFA rich fish oil diets on days –7, 20, 38 and 55 of supplementation.

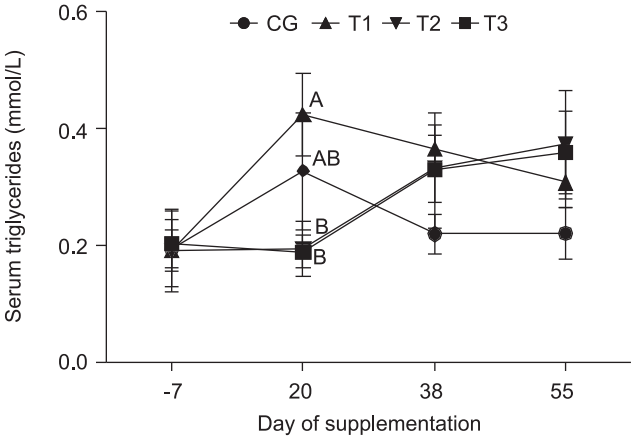


Fig. 2. Serum triglycerides concentrations (mmol/L) in goats fed different levels of n-3 PUFA rich fish oil diets on days –7, 20, 38 and 55 of supplementation. A and B indicate significant (P < 0.05) difference between the groups.

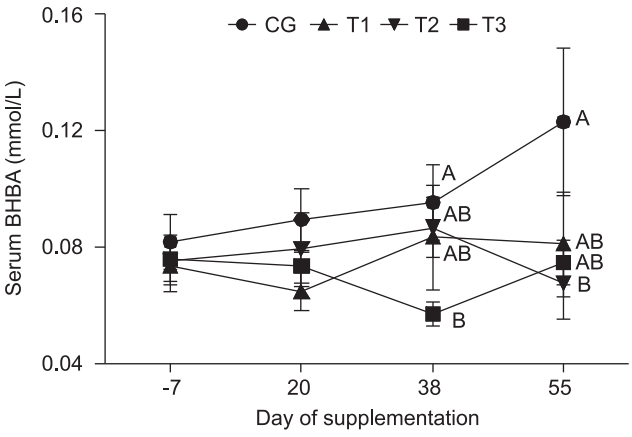


Fig. 4. Serum BHBA concentrations (mmol/L) in goats fed different levels of n-3 PUFA rich fish oil diets on days –7, 20, 38 and 55 of supplementation. A and B indicate significant (P < 0.05) difference between the groups.

supplementation than T1; however, no significant differences could be noticed in TGs compared to CG (Fig. 2). A decrease in plasma triglycerides concentration has been recorded with n-3 fatty acid supplementation in rat (Fickova *et al.* 1998) and humans (Christensen *et al.* 1999). On the other hand, plasma triglycerides were not significantly affected in cows and buffaloes supplemented with saturated fat (Hightshoe *et al.* 1991, Lammoglia *et al.* 1996) and fish oil or meal (Childs *et al.* 2008b, Malik *et al.* 2013). It is possible that the circulating triglycerides concentration may be affected by either the duration of fat supplementation or type and proportion of saturated or unsaturated fatty acid in the diet.

There was no significant day effect on serum NEFA concentration during the the 56 d of n-3 PUFA supplementation (Fig. 3) which is supported by the results of Robinson *et al.* (2002) and Childs *et al.* (2008b). The mean BHBA concentration was lower ($P<0.05$) in T3 than CG on day 38 of supplementation (Fig. 4); on day 55 of supplementation, it was lower in T2 ($P<0.05$) and T3 ($P<0.1$) than the CG. In addition, a treatment and day interaction ($P<0.05$) was observed on serum BHBA. The effect of n-3 PUFA supplementation on BHBA concentration is inconclusive as some authors reported no change (Ruppert *et al.* 2003), while others reported either an increase (Childs *et al.* 2008b) or decrease (Childs *et al.* 2008a).

According to Thomas *et al.* (1997), the considerable variation in the effects of fat supplementation on the metabolites and metabolic hormones may be explained by the degree of saturation and length of fatty acids present in the fat source. Short chain fatty acid esters in fats are hydrolyzed and biohydrogenated with very high efficiency than saturated fatty acid, whereas, fatty acid esters of long chain PUFA such as EPA and DHA are hydrolyzed least efficiently and tend to pass through the rumen unmodified (Byers and Schelling 1988). This may alter the fermentation pattern of fatty acids in the rumen and their absorption in the intestine to influence the serum biochemical profile.

In conclusion, medium and high dose n-3 PUFA rich fish oil reduced serum cholesterol in the goat by 55 day of supplementation.

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