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Effect of fish meal supplementation on production and biochemical alterations in dairy buffaloes during early postpartum period

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ABSTRACT

Objective: To assess the effects of supplementary feeding of fish meal on body condition score, milk production and associated blood biochemical alterations if any, in dairy buffaloes during early postpartum period. **Methods:** Ten pluriparous buffaloes belonging to organized dairy farm were supplemented with 250 g fish meal (FM) daily from day of calving for 90 days postpartum and 5 buffaloes were kept as unsupplemented control. Heparinized venous blood samples were collected from each buffalo on day 0 and 15 days interval thereafter till 75 days postpartum. Body condition score and milk yield was recorded at fortnightly and weekly intervals respectively. Blood samples were analyzed for glucose, total protein, cholesterol, BUN and triglycerides. **Results:** The fish meal supplementation did not affect the body condition score (2.90 ± 0.08 *vs.* 2.92 ± 0.10 and 2.31 ± 0.04 *vs.* 2.25 ± 0.09, at start and end of fish meal supplementation in supplemented and control buffaloes respectively) or the average milk production (3.30 ± 0.16 kg *vs.* 3.5 ± 0.17 kg in supplemented and control buffaloes respectively) significantly. The blood glucose and cholesterol concentrations increased during postpartum period (till 75 days postpartum) in both the groups and in a similar manner. Plasma blood urea nitrogen concentrations were significantly reduced in both groups in postpartum period ($P<0.05$). Plasma triglycerides varied non-significantly during postpartum. **Conclusions:** The fish meal supplementation does not affect the observed biochemical parameters. From the study, it can be concluded that fish meal supplementation (250 g daily) do not alter either biochemical profiles or milk production and body condition of the buffaloes.

1. Introduction

Buffaloes hold immense socio-economic importance to marginal farmers in several developing countries. However, their reproductive performance is very poor due to their inherent susceptibility to environmental stress which causes anestrus and subestrus [1]. Subestrus or silent estrus constitutes the largest factor responsible for poor reproductive efficiency in buffaloes [2]. There is high incidence of subestrus and anestrus especially in early postpartum period, resulting in prolonged calving interval and huge economic losses to the dairy industry. Delayed onset of postpartum ovarian activity in dairy cattle is attributed to various factors including nutrition, management, season, infection and genetic make up. Among these, nutrition is a major factor controlling length of postpartum anoestrus period [3]. Negative energy balance (NEB) decreases circulatory concentrations of glucose, insulin and insulin like growth factor (IGF-I), leading to increased losses of body condition score (BCS) and higher incidence of anoestrus in cows [4]. Fat supplementation began as a method of increasing dietary energy density in an effort to ameliorate negative energy balance in lactating postpartum cattle[5]. Fish meal (FM) used in dairy cow rations as a source of RUP also contains a relatively high

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concentration of two polyunsaturated fatty acids (PUFA) of the n-3 family, eicosapentaenoic acid (EPA, C20;5) and docosahexaenoic acid (DHA, C22;6). The studies on the effects of FM supplementation in buffaloes are obscure. Hence the present study was undertaken to evaluate certain biochemical profiles in PUFA (FM) supplemented feeding in postpartum buffaloes.

2. Materials and methods

The study was carried out on 15 postpartum Murrah buffaloes maintained at dairy farm, Kapurthala (Punjab). All the buffaloes had normal calving and subsequent normal genital health as assessed gynaeco-clinically. All the animals taken were in 2nd to 5th parity having an average body weight of 350-450 kg. The animals were housed under semi-loose housing system and fed 10 kg of green fodder (millet, maize or sorghum), 2-3 kg wheat straw and 1-2 kg of concentrate daily. The buffaloes were milked twice a day and were provided with *ad lib*. fresh drinking water thrice daily. The buffaloes were randomly divided into two groups. Postpartum buffaloes were kept on normal routine feeding without FM supplementation (Group I, n=5). Diet in 10 postpartum buffaloes were supplemented with 250 g/head/day FM daily for 90 days postpartum. Initially the FM was fed 100 g/head/day for the first week postpartum (acclimation period; Group II, n=10). On dry matter basis, FM contained 25.40% crude protein, 6.29% crude fibre, 5.22% ether extract and 64.13% ash. The total lipid content (5.86%) and free fatty acids (1.06%) of FM was determined as per the methods described by Folch *et al.* [6] and Lowry and Tinsley [7] respectively.

Blood samples (10 mL) were collected from all animals in heparinized polystyrene tubes (1:1000) on day of calving and then at 15 days interval till 75 days postpartum. Blood (5 mL) was collected separately for glucose estimation in glass vials having sodium fluoride (2.5 g/mL). The blood samples were placed in ice and centrifuged with in 2-3 h of collection, plasma separated by centrifugation was stored at -20 °C pending biochemical analysis for total protein, cholesterol, BUN and triglycerides. Glucose estimation was done within 48-72 h of blood collection. The biochemical profiles (glucose, total protein, cholesterol, BUN, triglycerides) were determined in plasma samples by using standard kits and an autoanalyzer (Microlab-300, Merck specialities Pvt Ltd). Fortnightly body condition scoring (Linear scale 1-5) was done from upto 75 days postpartum using body condition score chart described by Edmonson *et al*[8]. Average weekly milk yield of experimental animals was recorded for the 60 days postpartum. The data obtained was analyzed statistically by students t-test and DMRT [9] using statistical software version SPSS-16.

3. Results

The changes in various blood biochemical parameters obtained on different days postpartum in both groups are presented in Table 1. In both the groups, there was low blood glucose concentration at parturition. The concentration of blood glucose during postpartum increased gradually by day 15 post partum and there after. There was significant (P<0.05) difference in plasma glucose levels measure on day 0 and day 75 postpartum in both treatment group (52.25±2.07 mg/dL *vs.* 63.20 ±2.06 mg/dL) and control group (53.08±2.90 mg/dL *vs.* 66.28±2.23 mg/dL) respectively. PUFA supplementation did not cause any significant change (P<0.05) in blood glucose levels. Plasma protein concentration on day of calving in control group was (7.78±0.04) g/dL and (7.92±0.07) g/dL in FM supplemented buffaloes (Table 1). The protein concentration declined non-significantly on 15 day postpartum and there after it showed a gradual increase till day 75 postpartum. Plasma total proteins values on different corresponding days postpartum showed non-significant difference between treatment and control groups. The plasma cholesterol concentration was lowest in both treatment (57.36±2.94) mg/dL and control group

Table 1
Blood biochemical parameters (mean±SE) of PUFA supplemented and control buffaloes.

Parameter	Group	Days postpartum					
		0	15th	30th	45th	60th	75th
Glucose (mg/dL)	Treatment	52.25±2.07^{a}	58.38±1.56^{ab}	61.06±2.91^{b}	62.69±3.41^{b}	63.14±2.82^{b}	63.29±2.06^{b}
	Control	53.08±2.90^{a}	56.48±2.44^{ab}	59.54±3.22^{abc}	65.50±2.99^{bc}	62.88±2.58^{bc}	66.28±2.23^{c}
Total protein (g/dL)	Treatment	7.92±0.07^{a}	7.41±0.17^{a}	7.68±0.24^{a}	7.95±0.16^{a}	8.20±0.31^{a}	8.36±0.19^{a}
	Control	7.78±0.04^{b}	7.20±0.36^{b}	7.56±0.38^{b}	7.88±0.18^{b}	8.14±0.44^{b}	8.28±0.235^{b}
Cholesterol (mg/dL)	Treatment	57.36±2.94^{a}	65.34±3.88^{ab}	70.12±3.76^{ab}	70.47±2.03^{ab}	71.10±2.69^{b}	76.60±3.21^{b}
	Control	57.90±3.75^{a}	62.20±2.95^{ab}	71.50±3.51^{bc}	73.60±1.99^{bc}	73.60±3.13^{bc}	74.14±3.09^{bc}
BUN (mg/dL)	Treatment	19.26±1.15^{a}	17.10±0.99^{ab}	15.68±0.56^{ab}	15.04±0.95^{abc}	13.69±1.57^{abc}	13.50±1.7^{abc}
	Control	18.88±1.50^{a}	17.90±2.25^{ab}	16.24±1.02^{ab}	15.88±1.77^{ab}	15.40±2.6^{ab}	13.04±2.64^{b}
Triglyceride (mg/dL)	Treatment	16.02±1.82^{a}	13.77±1.20^{a}	16.0±1.27^{a}	16.82±1.91^{a}	13.94±1.63^{a}	14.43±1.48^{a}
	Control	14.140±2.74^{a}	16.02±1.56^{a}	12.90±1.73^{a}	13.12±1.62^{a}	14.10±1.27^{a}	15.30±1.27^{a}

In rows mean values having different superscripts differ significantly (P<0.05), corresponding values between supplemented and control groups were non-significantly different (P<0.05).

(57.90±3.75) mg/dL buffaloes at calving. After caving there was continuous rise in cholesterol levels till day 75 postpartum. Significant difference (P<0.05) existed in cholesterol levels at day 0 postpartum and at day 75 postpartum in both treatment (57.36±2.94 *vs*.76.60±3.21 mg/dL) and control (57.90±3.75 *vs*. 74.14±3.09 mg/dL) groups (Table 1). Dietary treatment showed non-significant effect on plasma cholesterol concentration in supplemented postpartum buffaloes.

Plasma BUN levels were significantly reduced (P<0.05) in both supplemented (19.26±1.15 *vs*. 13.50±1.70) and control buffaloes (18.88±1.50 *vs*. 13.04±2.64) almost in similar manner during postpartum period. There was non-significant effect of fish meal supplementation in BUN levels in treatment buffaloes as the BUN values at the corresponding stage in control group were non-significantly different. The fortnightly plasma triglyceride concentrations varied non-significantly between days postpartum. Dietary treatment had no effect on the plasma triglyceride concentration in fish meal supplemented buffaloes (Table 1).

At calving, the average BCS in control group and treatment group were (2.920±0.107) and (2.905±0.082) respectively. There was a continuous decrease in body condition score from calving to 75 days postpartum in both the groups. There was no significant difference in BCS between FM supplemented conceived and non conceived groups. The average weekly milk yield during first week in control and treatment buffaloes was (3.50±0.17) kg and (3.50±0.16) kg, respectively. In both the groups, there was a gradual and significant (P<0.05) increase in milk production during first 4 weeks postpartum. There after, it was almost constant for both groups. The milk production was unaffected by experimental diets.

4. Discussion

To the authors knowledge this is among the first studies to investigate the effect of FM supplementation on production and biochemical profile in dairy buffaloes during early postpartum period. High utilization of energy near calving for the impending parturition and diversion of energy for lactational purpose are the possible reasons ascribed for the decline in blood glucose concentration at parturition [10] as was seen in the present study in both the groups. There was increase in blood glucose concentration by day 15 post partum and thereafter. A gradual and significant increase (P<0.05) of blood glucose concentration was reported in buffalo by Quayam *et al*. [11] from day 5 to day 60 postpartum. The blood glucose level postpartum is related with the onset of cyclicity postpartum. Kabir *et al*. [12] found that postpartum cyclic buffalo had higher concentration of blood glucose than postpartum acyclic buffalo (73.1 mg/dL *vs*. 60.7 mg/dL). In present study both the treatment and control group buffaloes were having blood glucose levels less than 70 mg/dL by day 60 postpartum which might be the reason for buffaloes not showing signs of estrous. This suggests that buffaloes with significantly lower blood glucose concentration might become anestrous. PUFA supplementation did not cause any significant change in blood glucose levels. In agreement to our study Petit *et al*. [13] found no response in systemic glucose in postpartum cows fed with fish oil supplements. Grummer and Carroll [14] concluded that fat supplementation did not routinely increase blood glucose and stable blood glucose concentration during fat supplementation might indicate a reduction in hepatic gluconeogenesis. Heravi Moussavi *et al*. [15] observed an increase in blood glucose concentration in early lactating dairy cows fed fish meal. On other hand, Mattos *et al*. [16] reported decrease in plasma glucose levels in periparturient Holstein cows fed fish oil. Besides there was non-significant difference in plasma glucose concentration of conceived and non-conceived buffaloes at any of the intervals postpartum. However, the plasma glucose concentration was non significantly higher in conceived buffaloes as compared to those which did not conceived. This is concomitant with findings of Kavani *et al*. [17] who found a higher plasma glucose levels in postpartum buffaloes with fertile estrous cycle compared to those with infertile estrous cycle.

The plasma protein concentration declined non-significantly on 15 day postpartum and there after it showed a gradual increase till day 75 postpartum. These findings are in agreement with Lone *et al*. [18] who reported that total plasma proteins increased suddenly around calving, declined rapidly one week after calving and increased gradually thereafter. They suggested that sudden increase around calving and sudden decrease one week after calving in total plasma proteins could be due to increase in immunoglobulin fraction and reduction in albumin content in blood respectively. Quayam *et al*. [11] reported that the plasma concentration of total proteins is increased gradually from day 15 to day 60 postpartum in both pluriparous and primiparous buffaloes. Plasma total proteins values on different corresponding days postpartum showed non-significant difference between treatment and control groups. It indicates that there is no effect of dietary treatment on plasma protein concentration. However, no literature was available to substantiate the present findings of total proteins levels with respect to the FM supplementation. The difference in plasma total protein values were non significant between conceived and non-conceived groups. However, the plasma total protein values were non-significantly higher in conceived group compared to non conceived group. Shah *et al*. [19] also found higher total protein concentration in fertile buffaloes compared to infertile buffaloes.

The low concentration of plasma cholesterol at calving in both groups in the present study may be due to depletion of cholesterol reserves, as cholesterol is the precursor of steroids which increases considerably near the parturition stage [20]. These studies are consistent with findings of Bahga *et al*. [21] who reported that plasma cholesterol concentrations

were lowest around calving and there after increased linearly. Nath *et al.* [22] found that plasma concentration of cholesterol gradually declined with advancing pregnancy and gradually increased with advancing lactation. Dietary treatment showed non-significant effect on plasma cholesterol concentration in supplemented postpartum buffaloes. Petit *et al.*[13] and Lammoglia *et al.*[23] also concluded that there was non-significant effect of fish meal supplementation on the plasma cholesterol concentration. Contrarily, Thomas *et al.*[24] and Grummer and Carroll[14] observed that fish meal supplementation consistently increased the plasma cholesterol concentration. There was no significant difference in plasma cholesterol concentration between conceived and non conceived groups at any of the intervals postpartum. The findings of plasma cholesterol in conceived and non conceived groups were in agreement with Patel and Dhami[25].

Plasma BUN levels are influenced by amount of crude protein intake, degradability of protein energy intake and time of sampling post-feeding [26]. Excessive levels of crude protein in diet elevated BUN levels, altered uterine pH and reduce fertility in dairy cows [27] and buffaloes [28]. Reduction in plasma BUN levels significantly during postpartum period in present study are supported by the findings of Gadhave *et al.* [29] who reported that blood urea levels were highly increased at calving and declined during early postpartum period in crossbred cows. The marked increase in BUN levels at parturition could be associated with high muscle catabolism [29]. The PUFA supplementation did not appear to produce any harmful effect in terms of BUN levels as they were maintained with in physiological limits. Mattos *et al.* [30] and Heravi Moussavi *et al.* [15] also observed no effect of fish meal supplementation on plasma urea concentration in periparturient Holstein cows. Contrary to our findings, Childs *et al.* [31] reported an increase in plasma urea concentrations due to fish oil supplementation in beef heifers. Besides this, there was non significant difference in plasma BUN levels of conceived and non conceived buffaloes at any of the intervals postpartum. The plasma profile of triglycerides reflects lipid metabolism particularly in mammary glands of ruminants[32]. The non-significant variation in plasma triglyceride concentrations reported in present study are in agreement with observations of Patel and Dhami, [25] who found no specific variation in triglyceride in postpartum Holstein cows. Lack of effect of diet on systemic triglycerides is consistent with findings of Childs *et al.* [31] and Lammoglia *et al.* [33], who found no effect of fish meal /fish oil on plasma triglyceride levels in cattle. Besides this, there was no significant variation in plasma triglycerides between FM supplemented conceived and non conceived buffaloes. Patel and Dhami, [25] reported similar findings regarding values between conceived and non-conceived. The plasma profile of triglycerides was found to have significant negative correlations with plasma progesterone, glucose and total cholesterol [34].

There was no effect of dietary treatment on the BCS in supplemented buffaloes. Heravi Moussavi *et al.* [15], also reported that there is no effect of fish meal supplementation on BCS in lactating dairy cows. There was a significant decline in BCS in both treatment and control buffaloes. More than 70 percent buffaloes experience NEB during immediate postpartum period. Poor BCS associated with NEB resulted in higher incidence of anestrous [27], which might explain that all the animals supplemented as well as non supplemented did not showed estrous signs till day 60 postpartum. The milk production was unaffected by experimental diets. Abu-Ghazaleh *et al.* [35] and Mattos *et al.* [30], also found no beneficial effect of fish meal supplementation on milk yield. Contrary to it Heravi Moussavi *et al.* [15], observed increased milk yield by adding fish meal to diets. Variation in milk response to fish meal supplementation may be related to forage to concentrate ratio, stage of lactation and diet composition [35].

From the present study it was concluded that improvement of nutritional status by FM supplementation was not reflected through changes in biochemical profile and there was no significant effect of FM supplementation on BCS and milk yield.

Conflict of interest statement

We declare that we have no conflict of interest.

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