1. INTRODUCTION

In early lactating buffaloes, the amount of energy required for maintenance of body tissues and milk production often exceeds the amount of energy available from the diet (Goff and Horst, 1997), thus forcing mobilization of body fat reserves to satisfy energy requirement. Simultaneously, daily nutrient intake is insufficient to meet demands for milk production and energy balance is negative (Bell *et al.*, 1995). Due to depressed feed intake at the end of gestation, the period of negative energy balance often starts prior to calving. The negative energy balance in early lactation affects peak milk yield and overall lactation yield apart from causing delayed post-partum ovarian activity (Garnsworthy and Webb, 1999). The level of non-esterified fatty acids (NEFA) increases in plasma as a consequence of body fat mobilization and leads to hepatic lipidosis.

Calcium salts of long-chain fatty acids have been shown to be effective as ruminally inert fat supplements for lactating cows (Grummer, 1995) and is a good source for increasing energy density of the diet to improve productive performance. However, previous reports indicate that feeding of fatty acid (FA) supplements including calcium salts of palm FA linearly decreases dry matter intake (DMI) with increasing dietary concentration, whereas non-hydrogenated FA had no effect on DMI (Christensen *et al*., 1994).

The performance of lactation in dairy animal depends on the balanced feeding during postpartum period. It has been found that hydrogenated palm oil triglyceride provide a better energy supply for high-yielding dairy animals in negative energy balance than calcium soaps of palm oil fatty acids around calving. Prill fat is a non-hydrogenated vegetable oil and contains more than 85% palmitic acid with high melting point. Due to this reason, it does not melt at low pH, by pass rumen degradation and is digested in small intestine by lipase enzyme. Prill fat is prepared by liquefying mixture of fatty acid and spraying it under pressure into a cooled atmosphere. Prill fat remains inert in the rumen and resist hydrolysis and association with the bacterial cells of feed particles. Thus, total supplemented energy in diet of a lactating animal is available for the productive processes (Singh *et al*., 2014).

Choline, a component of phospholipid and methyl donor, plays an essential role in very low density lipoprotein synthesis and thereby contributes to fat export from the liver. Fat metabolism can be improved with the help of choline for better energy production. This also helps in improving milk production. Evidence suggests that the dietary supply of choline in early lactating dairy animals may be inadequate, even though choline can be synthesized by the animals (Pires and Grummer, 2008). As dietary choline gets degraded rapidly in the rumen, it must be supplemented in the protected form (Elek *et al*., 2008). Therefore, rumen protected form of choline has been developed to deliver choline to the small intestine for absorption (Garg *et al*., 2012b).

Considering the importance of prill fat as an energy source in diet of lactating animals and choline plays an essential role in fat metabolism the present experiment has been plan to find out the effect of rumen protects fat and choline supplementation in the diet of Murrah buffaloes with the following objective:

1. To study the effect of prill fat and protected choline supplementation on milk yield and its composition in Murrah buffaloes.

2. To study the effect of prill fat and protected choline supplementation on blood metabolic profile in Murrah buffaloes.

3. To study the effect of prill fat and protected choline supplementation on economics of milk production in Murrah buffaloes.

2. REVIEW OF LITERATURE

Present study was conducted to study the effect of prill fat and protected choline supplementation on performance, blood metabolic profile and economics of production in Murrah buffaloes. The work related to study was reviewed and is presented in this chapter.

2.1 Effect of prill fat supplementation on performance of dairy animals

Barley and Baghel (2009) studied the effect of bypass fat supplementation on performance of Murrah buffaloes. They observed that bypass fat supplementation increased average milk yield and fat content of supplemented buffaloes. They concluded that increased energy supply to the animals in negative energy balance was responsible for increase in milk yield.

Ganjkhanlou *et al*. (2009) conducted an experiment in Holstein cows (26±4 days in milk) were divided in three treatment groups: control (no fat supplementation) and supplemented with 30g prill protected fat (Energizer-10) or 35g Ca salt of protected fat (Magnapac) per kg feed. Cows were fed ad libitum a total mixed ration consisting of corn silage, alfalfa hay and concentrate mix. They found that intakes of dry matter (DM), organic matter (OM) and neutral detergent fibre (NDF) was decreased significantly with supplementation of rumen protected fat in cows ($p<0.05$), and milk efficiency (ECM/DM intake) was improved from 1.35 for control to 1.47 and 1.44 with supplementation of Energizer-10 and Magnapac, respectively.

Sirohi *et al.* (2010) conducted an experiment in which lactating crossbred cattle (n=10) were randomly divided in 2 groups on the basis of milk yield (14–15 kg/day), day of calving (~50 days) to see the effect of bypass fat supplementation on nutrient utilization and production performance. Cows in control group were fed wheat straw, concentrate mixture and green maize fodder whereas additional 300 g bypass fat was given in treatment group. Experimental feeding was continued up to 90 days after 2 weeks of adaptation. The average milk production, fat corrected milk, Milk fat and total solids yield per day was significantly higher in treatment group, whereas milk protein and solids-not-fat remained unaffected in both the groups. Intake per

kg of milk produced was lower in treatment group than the control group. Milk production efficiency was also significantly higher (30.22%) in bypass fat supplemented group as compared to the control group (27.03%).

Garg *et al.* (2012a) studied the effect of bypass fat supplementation on performance of crossbred cows. In addition to basal ration, cows in group II were fed 100 g bypass fat supplement, whereas, cows in group III were fed 100 g bypass fat and 10 g rumen protected choline (RPC) supplement per animal per day. Average increase in daily milk yield and fat in groups II and III over a 90 days experimental period were 1.48 kg ($p<0.01$) and 0.54% ($p<0.05$) and 1.77 kg ($p<0.01$) and 0.61% ($p<0.05$), as compared to control group. There was improvement ($p<0.01$) in milk poly-unsaturated fatty acid level in groups II and III. Total unsaturated fatty acids were also increased by 15.29 and 15.71% in groups II and III, respectively. They inferred that supplementing bypass fat helps improving milk and fat yield in crossbred cows, which can further be enhanced by fortification with rumen protected choline chloride.

Rajesh *et al*. (2014) conducted an experiment to study the effect of prilled fat feeding on milk production, feed intake and plasma hormones in cows during early lactation maintained as per routine management practices (control group) or in addition were fed prilled fat @75 g/d for a period of 90 days (supplemented group). They observed that plasma glucose levels were similar in both the groups ($p>0.05$), however NEFA level was decreased ($p<0.05$) in the prill fat supplemented group. Further, milk yield was increased ($p<0.05$) in prill fat supplemented group than the control group. Milk fat was increased by 9% in the supplemented group however protein, lactose and SNF remained unaffected. They also observed significantly ($p<0.05$) higher ether extract digestibility in prill fat supplemented group without any effect on dry matter intake, milk composition and plasma hormone levels.

Singh *et al.* (2014) conducted an experiment to find out the effect of prill fat feeding on milk production and hormonal changes in crossbred cows during mid lactation (150 days). During the experimental

period, cows were fed bypass prill fat @ 75 g/d (PFG) for a period of 90 days. The DMI and body weight of cows were non-significant ($p>0.05$) between the groups, but BCS of cows was improved in the control group. Milk yield was significantly ($p<0.05$) lower in control group over the prill fat feeding group cows. Milk fat, protein, lactose and cholesterol were similar in both the groups. They concluded that prill fat supplementation can be used to augment milk production without influencing milk composition.

Yadav *et al.* (2015) conducted research study to assess the effect of prill fat supplementation on milk production in Karan Fries cows. The animals in control group were fed as per requirements while treatment group received prill fat @ 75 g/cow/day in addition to control from 45 days prepartum till parturition. After parturition the treatment group received prill fat @ 150g/cow/day till day 70^{th} of lactation. They observed that body condition score of prill fat supplemented cows was better ($p<0.05$) in comparison to control group. Cows in treatment group produced more milk with higher fat and SNF contents ($p<0.01$) than control. Milk cholesterol concentration was significantly reduced ($p<0.01$) in animals of the treatment group.

Sharma *et al.* (2016) studied the effect of supplementation of prill fat in advance pregnant Murrah buffaloes. Buffaloes either received a dietary supplement of prill fat at 100 g/day for 35 days pre-partum and at 150 g/day for 95 days post-partum (supplemented group) or did not receive fat supplement (control group). They reported no any significant change in DMI between groups and periods of study. BCS of buffaloes was significantly ($p<0.01$) improved in the supplemented group than control group ($p<0.01$). Milk yield was higher ($p<0.01$) in supplemented group than in control group during 95 days of early lactation. Milk fat and fat corrected milk yield was significantly higher ($p<0.01$) in supplemented group however protein, lactose and solid not fat content did not varied between the groups. The feed efficiency of the supplemented group was higher ($p<0.01$) than the control group during the post-partum period. They inferred that prill fat supplementation augments the energy balance and milk production in transition Murrah buffalo.

2.2 Effect of protected choline supplementation on performance of dairy animals

Erdman and Sharma (1989) conducted two experiments to test the effects of graded amounts of rumen-protected choline on milk yield and composition in lactating dairy cows. In first experiment, 48 Holstein cows were fed 0, 0.078, 0.156, and 0.234 % rumen-protected choline (choline chloride basis) from week 5 to 21 postpartum. They observed that increasing choline level had no effect on DMI whereas and milk yield tended to increase only from 1.0 to 2.2 kg/d. Milk fat percentage was reduced in the 0.078% choline supplemented group, it was increased in control group followed by 0.156 and 0.234% choline supplemented group. In second experiment, 16 Holstein cows in mid lactation were assigned randomly to either 13.0 or 16.5% dietary CP (DM basis). Within CP concentration, cows were fed 0, 0.08, 0.16, and 0.24% rumen-protected choline in a replicated 4 x 4 Latin square design. Results of the experiment revealed that increasing dietary choline to 0.24% linearly increased milk yield 2.6 kg/d, although it had no consistent effects on milk fat or protein percentage. There was only a slight tendency for greater responses in milk yield to dietary choline with lower dietary CP. On the basis of the findings of experiments, they concluded that choline might be a limiting nutrient for milk production.

Lima *et al*. (2007) studied the effects of feeding rumen-protected choline (RPC) on lactation and metabolism in dairy cows. In experiment 1 (E1), 369 cows were fed 15 g/d of RPC from 25 d prepartum to 80 d. In experiment 2 (E2), 578 primigravid cows were fed 15 g/d of RPC in the 21 d prepartum. Prepartum DM intake was similar ($p>0.15$) between treatments and averaged 12.5 and 10.5 kg/d for E1 and E2, respectively, but intake tended ($p=0.10$) to be greater for cows fed RPC (23.9 vs 22.6 kg/d) in E1. In E1, yields (kg/d) of 3.5% FCM (44.6 vs 42.8), ECM (40.1 vs 38.5), and milk fat (1.61 vs 1.52) were greater ($p<0.05$), and of milk (43.1 vs 42.1) and true protein (1.21 vs 1.17) tended ($p=0.08$) to be greater for RPC than control. In E2, milk yield tended ($p=0.07$) to be greater for RPC than control (28.7 vs 27.9 kg/d). Body condition was improved ($p=0.01$) in postpartum dairy cows in E1.

Elek *et al.* (2008) conducted an experiment on Holstein cows during preparturient period to study the effect of rumen-protected choline (RPC) supplementation on performance and milk choline content Cows were fed the experimental diet from 21 days before expected calving until 60 days of lactation. They observed that milk yield was increased by 4.4 kg whereas 4% fat-corrected milk production was also increased by 2.5 kg/day in the group of cows receiving supplementary choline during the 60 days experimental period. Milk fat content was not altered by treatment, but fat yield was increased by 0.10 kg/day as a consequence of higher milk yield in the rumen protected choline treated group. Milk choline content was increased in both the groups after calving as the lactating period advanced. However, milk choline content and choline yield were significantly higher in the rumen protected choline group than in the control group. They concluded that improved milk choline and choline yield must be due to the reason that some of the applied rumen protected choline escaped ruminal degradation, was absorbed from the small intestine and improved the choline supply of the cows and contributed to the changes of production variables.

Sales *et al.* (2010) conducted a meta-analysis to quantify the effects of dietary rumen-protected choline on production characteristics of dairy cows. Milk yield was decreased marginally from 131.5 to 0.037 g of milk/g of dietary rumen-protected choline chloride when supplementation increased from 6 to 50 g/d. Milk fat content was decreased linearly at a rate of 0.00339% for a 1g/d increase in dietary rumen-protected choline chloride.

Zom *et al*. (2011) studied the effects of a dietary supplemention of rumen-protected choline in periparturient dairy cows. Animals were divided in two groups supplemented either with or without (control) rumen-protected choline. They observed that choline supplementation increased DM intake from 14.4 to 16.0 kg/d. Choline supplementation had no effect on milk yield, milk fat yield and lactose yield. Milk protein yield was increased from 1.13 to 1.26 kg/d at the intercept of the lactation curve at 1 DIM, but the effect of choline on milk protein yield was gradually decreased during the course of the study. Choline supplementation was associated with decreased milk fat concentration at the intercept of the lactation curve at 1 DIM, but the effect of

choline on milk fat concentration gradually decreased as lactation progressed. Choline supplementation had no effect on energy-corrected milk yield, energy balance, body weight and body condition score. Choline supplementation decreased the concentration of liver triacyl glycerol during the first 4 week after parturition. On the basis of results of their study, they inferred that hepatic fat export in periparturient dairy cows is improved by choline supplementation during the transition period and this may potentially decrease the risk for metabolic disorders in the periparturient dairy cow.

Garg *et al.* (2012b) conducted an experiment on Jaffarabadi buffaloes (n=27) yielding 8-10 kg milk/head/day which were divided into three groups of nine each, based on milk yield, fat per cent and stage of lactation. All animals were fed similar basal diet, comprising 12-15 kg green jowar and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements. Buffaloes in Group 2 were supplemented daily with 150 g bypass fat per animal whereas buffaloes in Group 3 were supplemented with 15 g rumen protected choline chloride along with 150 g bypass fat. Average increase in milk yield and fat of Groups 2 and 3 were 1.26 kg ($p<0.05$) and 0.31% ($p<0.05$) and 1.55 kg ($p<0.01$) and 0.44% ($p<0.05$), respectively as compared to Group 1. They observed significant improvement in polyunsaturated fatty acid level in Groups 2 and 3. Total unsaturated fatty acids also increased by 9.24 and 9.95% in Groups 2 and 3, respectively. Their results indicated that supplementing bypass fat helps improving milk and fat yield, which can be further enhanced by fortification with rumen protected choline chloride.

2.3 Effect of prill fat and protected choline supplementation on blood metabolic profile of Murrah buffaloes

Grummer (1995) conducted an experiment to study the impact of changes in organic nutrient metabolism on feeding transition dairy cows. He reported increased plasma NEFA concentrations occur the 10 d before calving and was highest at calving and decrease rapidly after calving. Plasma glucose concentration decreases during the transition period except for a transient increase associated with calving.

Wang *et al*. (2004) conducted an experiment to investigate the effect of the diets supplemented with lard or prilled fat (Carolac) on performance and blood metabolic profile of Holstein cows under a warm climate. The treatments were basal diet (Control), basal diet supplemented with 2.5% lard (LD), and basal diet supplemented with 2.5% commercial Prilled fat (PF). The results indicated that the DM intake did not differ among the treatments. Milk yield and 4% FCM yield were greater ($p<0.05$) in PF than in Control. LD and PF resulted in greater milk fat percentage. Protein, lactose and solid contents in milk were not significantly different among the three dietary treatment groups. The concentration of non-esterified fatty acids (NEFA) in plasma was significantly greater in LD and PF than that in Control. However, the concentrations of triglycerides, urea nitrogen, and cholesterol in plasma were not significantly different among the three treatment groups.

Lima *et al*. (2007) studied the effects of feeding rumen-protected choline (RPC) on lactation and metabolism in dairy cows. In experiment 1 (E1), 369 cows were fed 15 g/d of RPC from 25 d prepartum to 80 d. In experiment 2 (E2), 578 primigravid cows were fed 15 g/d of RPC in the 21 d prepartum. Concentration of glucose tended to be greater ($p=0.10$) in E1 and of NEFA was smaller ($p=0.05$) at calving in E2 for RPC compared with control.

Barley and Baghel (2009) studied the effect of bypass fat supplementation on serum triglyceride content in Murrah buffaloes. They observed that bypass fat supplementation increased serum triglyceride content of supplemented buffaloes. They concluded that increased energy supply to the animals in negative energy balance was responsible for increase availability of low density serum triglyceride in plasma led to increased serum triglyceride levels.

Garg *et al.* (2012a) studied the effect of bypass fat supplementation on performance of crossbred cows. In addition to basal ration, cows in group II were fed 100 g bypass fat supplement, whereas, cows in group III were fed 100 g bypass fat and 10 g rumen protected choline (RPC) supplement per animal per day. Non-esterified fatty acids (NEFA) level in blood serum was reduced by 16.12 and 24.19% ($p<0.01$) in groups II and III, respectively. There was reduction ($p<0.01$) in cholesterol levels in blood

serum in animals of groups II and III, as compared to control group. Blood glucose and urea nitrogen were not affected by the dietary treatments.

Garg *et al.* (2012b) conducted an experiment on Jaffarabadi buffaloes (n=27) yielding 8-10 kg milk/head/day which were divided into three groups of nine each, based on milk yield, fat per cent and stage of lactation. All animals were fed similar basal diet, comprising 12-15 kg green jowar and 4-6 kg groundnut straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements. Buffaloes in Group 2 were supplemented daily with 150 g bypass fat per animal whereas buffaloes in Group 3 were supplemented with 15 g rumen protected choline chloride along with 150 g bypass fat. Non-esterified fatty acid level in the blood serum was decreased by 7.69 and 18.46% ($p<0.05$) in Groups 2 and 3, respectively. There was significant ($p<0.05$) reduction in the cholesterol levels in the blood serum of animals of Groups 2 and 3, as compared to Group 1.

Singh *et al.* (2014) conducted an experiment to find out the effect of prill fat feeding on blood metabolic profile of crossbred cows during mid lactation (150 days). During the experimental period, cows were fed bypass prill fat @ 75 g/d (PFG) for a period of 90 days. Plasma hormones namely growth hormone, triiodothyronine and thyroxin were significantly ($p<0.05$) lower in control group than PFG cows. Furthermore, plasma NEFA concentration was decreased ($p<0.05$) in PFG cows whereas glucose level varied non-significantly between the groups.

Yadav *et al.* (2015) conducted research study to assess the effect of prill fat supplementation on certain plasma metabolites in Karan Fries cows. The animals in control group were fed as per requirements while treatment group received prill fat @ 75 g/cow/day in addition to control from 45 days prepartum till parturition. After parturition the treatment group received prill fat @ 150g/cow/day till day 70^{th} of lactation. The concentration of plasma NEFA ($p<0.01$) and total cholesterol was reported lower ($p<0.05$) in prill fat supplemented cows than the control group.

2.4 Effect of prill fat and protected choline supplementation on economics of milk production in Murrah buffaloes

Naik *et al.* (2009) conducted a field trial to study lactation response of crossbred dairy cows fed on indigenously prepared rumen protected fat. The animals were offered concentrate mixture, green berseem and wheat straw daily as basal diet. During the whole experimental period, the animals in three groups were randomly supplemented without (control) or with 200 g rice bran oil (RBO200) or 200 g prill fat (PF200) on fat equivalent basis. They recorded net profit of Rs 34.50/cow/day in prill fat supplemented group over the control group during early lactation.

Mohsen *et al*. (2011) conducted a research trial to evaluate the effect of rumen protected choline supplementation on digestibility, rumen activity and milk yield in lactating friesian cows. Twelve lactating Friesian cows at 2^{nd} to 5^{th} lactating season, with body weight of 500±15 kg were fed a basal ration consisting of concentrate feed mixture, fresh berseem and rice straw supplemented with rumen-protected choline (RPC) in the form of choline chloride at levels of 0, 15 and 30 g/head/day. They observed 10.94 and 18.28 % increase ($p<0.05$) in average income of milk yield in 15 and 30 g RPC supplemented group compared with un-supplemented group, respectively.

Gowda *et al*. (2013) performed an experiment in field condition to evaluate the effect of protected fat supplementation in high yielding dairy cows. Twelve numbers of high yielding crossbred (Holstein Frisian) dairy cows in their 2-5th lactation maintained by farmers were selected based on their previous lactation yield. Soon after calving, the first group of cows were maintained on the existing feeding schedule practiced by the farmers (G I) and second group of cows were supplemented with protected fat (10g/lit milk) in addition to the existing feeding schedule for 195 days duration (G II). They inferred that feeding of protected fat resulted in a net profit of Rs. 11.60 per cow per day due to higher milk production suggesting that feeding protected fat was economically viable.

Singh *et al.* (2014) conducted an experiment to find out the effect of prill fat feeding on milk production and hormonal changes in crossbred cows during mid lactation. Cows were fed with bypass prill fat @ 75 g/d for a period of 90 days. Prill fat feeding incurred extra cost of Rs. 8/day/animal during the experimental period and generated additional income of Rs. 50/day/animal by increased milk and fat yield. The total income return from the prill fat fed cows was significantly ($p<0.05$) more than that of control group.

Yadav *et al.* (2015) have under taken a study to assess the effect of prill fat supplementation on economics of milk production in Karan Fries cows. The animals in control group were fed as per requirements while treatment group received prill fat @ 75 g/cow/day in addition to control from 45 days prepartum till parturition. After parturition the treatment group received prill fat @ 150g/cow/day till day 70^{th} of lactation. They concluded that feeding of prill fat was economical resulting in an additional income generation of Rs. 94.46/cow/day.

3. MATERIALS AND METHODS

Considering the importance of prill fat as an energy source in diet of lactating buffaloes and choline playing essential role in fat metabolism the present experiment has been planned to find out the effect of prill fat and protected choline supplementation in the diet of Murrah buffaloes.

3.1 Location of work

Proposed work was conducted at Livestock Farm, Adhartal, College of Veterinary Science and A.H., N.D.V.S.U., Jabalpur (M.P.).

3.2 Experimental animals and management

3.2.1 Experimental animals

Eighteen Murrah buffaloes (n=18) were divided into three similar groups (Control, T_1 and T_2) of six each, based on level of milk production, fat %, and stage of lactation (2-3 weeks post-partum). Buffaloes in all the three groups were fed a similar total mixed basal diet, comprising green fodder and wheat straw. Concentrate mixture was given according to the level of milk production to meet the maintenance and milk production requirements (Kearl, 1982). Buffaloes in Group T_1 were supplemented with prill fat @ 2.5% of total DMI per buffalo per day and while, buffaloes in GroupT_2, were supplemented with prill fat @ 2.5% of total DMI per buffalo per day and 54 g rumen protected choline in their basal ration.

All the experimental buffaloes were housed in a well-ventilated shed having cemented floor with individual feeding arrangement. The buffaloes were dewormed before start of the experiment and standard managemental practices were followed in the shed. In morning and evening, buffaloes were offered weighed quantity of concentrates followed by greenroughage feeding. The dry roughage (wheat straw) was available *ad-libitum*. The animals were let loose for about 1-2 hours daily in the surrounded paddock for exercise. Clean fresh water was made available *ad-libitum*.

3.2.2 Experimental diets

The basal diet (concentrate mixture along with green berseem and wheat straw) was formulated to meet all the nutrient requirements as per Kearl (1982) for buffaloes.

3.2.3 Dietary treatments

The feeding trial was conducted at the Livestock Farm, Adhartal (LSF) using Completely Randomized Design. For this purpose, eighteen lactating buffaloes were randomly allocated with 3 dietary treatments:

Control : Basal diet (was formulated using wheat straw, green berseem fodder along with concentrate and was fed as total mixed ration according to nutrient requirement of lactating buffaloes. During the experiment all the buffaloes in each treatment were fed similar basal diet, comprising 12-15 kg green berseem fodder and 8-10 kg wheat straw (Kearl, 1982).

T_1 : Basal diet**+** supplemented with prill fat @ 2.5% of total DMI per buffalo /day.

T_2 : Basal diet**+** supplemented with prill fat @ 2.5% of total DMI per buffalo /day**+**54 g rumen protected choline per buffalo /day.

3.3 Duration of experiment

The feeding trial was for a period of 3 months (November to February) with adaptation period of 2 weeks. The total experimental period was of 6 months.

3.4 Parameters to be studied

During the experimental period following observations were recorded:

3.4.1 Performance

3.4.1.1 Daily feed intake

All buffaloes were fed individually. The allocated amount of feed and fodder offered and left over was collected next morning from individual buffaloes and daily feed intake was recorded. Average daily feed intake of

each animal was calculated by measuring feed offered and residue left for three consecutive days with the help of spring balance.

3.4.1.2 Milk yield

Average daily milk yield of individual lactating buffaloes were recorded (morning + evening) and average milk production of individual animal was calculated on fortnightly basis.

3.4.1.3 Milk Composition

Milk samples were collected on monthly basis from each buffaloes in different treatments and analyzed for fat, solids-not-fat, milk protein, density and lactose by using Lacto scan.

3.4.1.4 Body Weight

Body weight of each animal under different groups was recorded at the start and at completion of experiment. It was done using platform balance for large animals.

3.4.1.5 Body condition score

The body condition of animals was estimated by using a simple visual technique called body condition scoring (BCS) developed by Edmonson *et al*. (1989) that gives a fairly high accuracy. A chart was prepared so that each area of the body that was considered important in assigning an overall body condition score was examined individually for changes along to 1 to 5 point scale using 0.5 unit increments. A score of one indicated an emaciated condition and a score of five indicated an obese condition. A total of eight areas of the buffalo body were examined and criteria within each area were used to indicate the body condition. The eight locations examined were in three major regions as detailed below:

A. Loin

1. Spinous processes (the vertical prominences of the lumbar vertebrae).
2. Depression between the spinous and transverse processes

3. Transverse processes (the transverse prominences of the lumbar vertebrae).

4. Overhanging shelf formed by the transverse processes above the flank.

B. Pelvis

5. Tuber coxae (hooks) and tuber ischii (pin bones) bony prominences

6. Depression between the hook and pin bones.

7. Depression between the hooks.

C. Tail head

8. Spinous and transverse processes of the coccygeal vertebrae and ischiorectal fossa (depression beneath the tail).

All BCS were assigned by same individual. Buffaloes were scored at start of experiment and thereafter fortnightly up to 120 days of lactation for body condition and the scorer had no knowledge of the previous BCS. The buffaloes were scored during the time of concentrate feeding after they were tied.

3.4.2 Blood metabolic profile

Collection of blood samples**:** During the study, blood samples (10 ml) were collected aseptically from all the buffaloes of different groups, firstly before the supplementation and subsequently after the three months of supplementation. Blood was collected from jugular vein aseptically in a sterilized plastic tubes containing anti-coagulant heparin solution (0.2 mg/ml of blood). Tubes containing blood samples were kept in ice (0ºC) and brought to the laboratory. In the laboratory, blood was centrifuged for separating the plasma.

Plasma samples were then analyzed for glucose, total cholesterol and triglycerides. Remaining plasma samples was stored in glass vials in a deep freeze (-20ºC) for further analysis of non-esterified fatty acids (NEFA).

3.4.3 Economics of production

Economics of milk production was calculated without and with supplementation study based on the expenditure on feeds and return from the milk production of buffaloes.

3.5 ANALYSIS

3.5.1 Chemical Analysis

3.5.1.1 Proximate analysis of feed and fodder

Different feed ingredients, green and dry fodder as well as concentrate mixture were analyzed to know the dry matter, crude protein, ether extract, crude fibre, nitrogen free extract and total ash content as per the methods described in the manual of Association of Official Analytical Chemist (AOAC, 2005).

3.5.1.1.1 Determination of dry matter (DM)

Representative samples were taken in previously weighed moisture cup/tin trays and kept in hot air oven at100±2°C for 24 hrs. Dry matter was calculated as follows:

$$\text{Dry matter}\,(\%) = \frac{b}{a} \times 100$$

Where, a = fresh weight of sample (g), b= dry weight of sample (g)

3.5.1.1.2 Determination of total ash (TA)

Five gram of air dry samples was taken in previously weighed silica crucibles. The crucibles along with samples were kept on heater and burnt till smoke disappears from charred mass of samples. With the of metal tong, the silica crucibles were transferred in to Muffle furnace and ignited at 600°C for 2 hours. After 12 hrs crucibles containing ash were removed from the furnace and transferred into desiccators, cooled and weighed. Total ash content was expressed on DM basis and calculated as follows:

$$\text{Total ash}\,(\%) = \frac{a - b}{c} \times 100$$

Where, a= weight of silica crucible with ash (g), b= weight of empty silica crucible (g), c= weight of dry sample taken for ashing (g)

3.5.1.1.3 Determination of nitrogen and crude protein (CP)

Nitrogen and crude protein in samples were estimated by using Kjeldahl method. The representative samples of ration and residue were digested in Kjeldahl flask with commercial sulphuric acid in the presence of digestion mixture ($CuSO_4$:K_2SO_4; 1:10). Then digested sample was transferred in to a volumetric flask to make a suitable volume of 250 ml, were cooled and out of which 25 ml sample was subjected to distillation in the semi-automatic Kjeltec distillation assembly. The ammonia released during distillation was collected in to 30ml of 2% per cent boric acid solution containing mixed indicator (0.2 per cent methyl red and 0.1 per cent bromo cresol green in equal amount in 95 per cent ethyl alcohol). The ammonia collected in boric acid solution was titrated against 0.1N HCL.

$$\text{Niitrogen}(\%) = \frac{(V1 - V2) \times 0.0014 \times \text{volume made}}{B \times \text{aliquot taken}} \times 100$$

Where,

V1 = Volume (ml) of 0.1N HCL used for titration of samples

V2 = Volume (ml) of 0.1N HCL used for titration of blank

B = Weight of sample taken for digestion on DM basis

0.0014= Molecular weight of nitrogen (g) equivalent to neutralize 1 ml of 0.1 N HCl.

Crude protein (%) = Nitrogen (%) x 6.25

3.5.1.1.4 Determination of ether extract (EE)

The ether extract content of the sample is estimated by extracting it with a fat solvent like petroleum ether. Ether is continuously volatilized at their boiling point condensed and allowed to pass through the sample in soxhlet apparatus. Thus, the ether extract obtained.

Weight the sample in to a weighed extraction thimble having porosity permitting rapid passage of ether. Remove the water from sample by placing it in hot air oven for overnight and cool it in desiccator and weigh.

Place the thimble in the soxhlet apparatus in straight direction so that the condensed ether may drop on it. Take out the thimble and keep it at room temperature for evaporation of ether and keep overnight in the oven at 100-105^{0}C. Remove the thimble from hot air oven, cool it in desiccator and weigh.

EE (%) = weight of thimble and sample before extraction- weight of thimble and sample after extraction x 100.

3.5.1.1.5 Determination of crude fibre (CF)

The samples after defatting above were transferred from thimble to spoutless beaker of one litter capacity and in each beaker, 200ml of 1.25 % H_2SO_4 was added. It was refluxed for 30 min. on hot plate after the boiling started and thereafter, filtered through muslin cloth. The residue was washed 5-6 times with hot water until it became acid free. The residual materials on the muslin cloth were again transferred to the respective beaker and in each beaker 200ml of 1.25% sodium hydroxide solution (NaOH) was added.

It was again refluxed for 30 min. after the boiling started and there after filtered through muslin cloth and washed with hot water for 5-6 times until became free from alkali. Thereafter, total residue was transferred in a clean dry silica crucible dry in hot air oven at 100±2°C for 24 hours and then it was cooled in desiccators and weighted.

The residue was then ignited in Muffle furnace at 600°C for 2 hrs after 12 hrs crucible containing ash were removed from the furnace and transferred in to desiccators, cooled and weighed again. Weight loss due to ignition was recorded as the weight of crude fibre.

$$\text{Crude fibre}\,(\%) = \frac{b}{a} \times 100$$

Where,

a= Weight of sample on dry matter basis (g)

b= Weight of crude fibre (g)

3.5.1.1.6 Determination of nitrogen free extract (NFE)

Nitrogen free extract (NFE) content in feeds and fodder was arrived at by subtracting the sum total of crude protein, ether extract, crude fiber and total ash per cent from 100.

Nitrogen free extract (%) =100 - (CP%+ EE% + CF% + TA %)

3.5.2 Milk analysis

Milk samples were collected on monthly basis from each animal in different treatments and analyzed for fat, solids-not-fat, total solid, milk protein, density and lactose by using Lacto scan.

3.5.2.1 Fat corrected milk

Four percent fat corrected milk (4% FCM) was calculated by formula of Gains (1928).

4% FCM (kg) = [(0.4 x total milk) + (15 x total fat)]

3.5.2.2 Energy corrected milk

Energy corrected milk (ECM) was calculated as per Tyrrell and Reid (1965).

ECM (kg) = [(7.2 x kg protein) + (12.95 x kg fat) + (0.327 x kg milk)]

3.5.3 Estimation of Blood biochemical parameters:

The blood samples from 2 buffaloes of each group were collected on monthly basis. After collection in EDTA, these samples were immediately centrifuged for 15 minutes at 5000 rpm at 4°C. The extracted plasma was used to analyze glucose, total cholesterol and triglycerides by using Semi-Automatic Blood Biochemical Analyzer with appropriate commercial kits.

3.5.3.1 Non-esterified fatty acids (NEFA) estimation:

The blood metabolite non-esterified fatty acids (NEFA) were estimated in plasma samples from buffaloes by using copper soap solvent extraction method modified by Shipe *et al.* (1980).

Principle

The proteins of plasma precipitated with hydrochloric acid and thus the fatty acids are free from proteins. The free fatty acids subjected to react with copper reagent to convert into copper soaps, which recovered by extraction with a chloroform heptane methanol solvent. The extracted copper soaps reacted with a colour reagent to develop a yellow colored compound, which is measured calorimetrically at 440 nm.

Preparation of reagents

1. **Copper reagents:** The copper reagent was a mixture of 5 ml of triethanol amine and 10 ml of 1M aqueous cupric nitrate [$Cu(N0_3)_2.3H_20$]. It was diluted to 100 ml with saturated sodium chloride (NaCI) solution. The pH was adjusted to 8.3 with 1N sodium hydroxide (NaOH) solution. The mixture was stored in the dark at room temperature, in order to ensure that the material remained stable for a period of at least 4-5 months.

2. **Colour reagent:** It was mixture of 0.5 percent sodium diethyl dithiocarbomate solution in n-butanol, i.e. 0.5 g per 100 ml.

3. **Solvent mixture:** Solvent mixture was consisting of chloroform, heptane and methanol (all GR grade) in the ratio of 49:49:2 (v/v), respectively and the mixture was designated, as CHM.

4. **Stock standard palmitic acid solution:** 5.12 g of pure palmitic acid was dissolved in 100 ml solvent mixture giving a solution of palmitic acid concentration of 0.2mol/L. From this solution, 1 ml was taken in a 100 ml with solvent mixture giving a final concentration of 2 mmol/L, or 2000 µmol/L.

5. **Working standard palmitic acid solution:** 2 ml of stock solution was first diluted with 10 ml of solvent mixture (5 times dilution) to obtain a solution of palmitic acid concentration of 400 µmol/L. This solution (400 µmol/L concentration) was then diluted serially to obtain a series of working standard solutions having the concentrations of 200, 100, 50, 25 µmolpaImitic acid / L.

Procedure

1) 0.1 ml of, 0.7N HCL was added to 0.5 ml plasma sample in duplicate in a 16x125 mm screw cap test tube. The mixture was shaken on a vortex test tube mixer. Simultaneously, 0.5 ml aliquots of working standard solutions having concentrations of 0 (blank) 25, 50, 100, 200 and 400 µmol palmitic acid/L were taken in another 6 screw cap test tube (16 x 125 mm).

2) Thereafter, 2 ml of copper reagent followed by 6 ml of the solvent mixture were added.

3) All the test tubes were shaken for 30 minutes on shaker at 240 R.P.M.

4) Then centrifuged for 10 minutes at 300 R.P.M.

5) 3.5 ml of the solvent layer was transferred to an acid washed test tube containing 0.1 ml of the colour reagent.

6) After mixing, the colour intensity (yellow colour) was measured at 440 nm within one hour using spectrophotometer (Model: Systronics 118) against blank. The standard curve was drawn for different concentrations of palmitic acid against optical density (OD) readings. Then the OD of samples was plotted against the standard curve and thus, the concentrations of NEFA in the samples were estimated and expressed as µmol NEFA/litre of plasma.

3.6 Statistical analysis

The data obtained in the study was statistically analyzed through analysis of variance technique under Complete Randomized Design (Snedecor and Cochran, 1994).

4. RESULTS

The present study was undertaken to study the effect of prill fat and protected choline supplementation on performance, blood metabolic profile and economics of production in lactating Murrah buffaloes. The data obtained during the study were analysed statistically and is presented in this chapter under following heads.

4.1 Proximate composition of feed ingredients

The proximate composition of various feed ingredients used in the study is presented in Table 01. The analysed proximate composition (% DM basis) of ingredients used to prepare concentrate mixture i.e. yellow maize, wheat bran, rice polish, arhar chuni, groundnut cake and mustard cake was as DM: 91.66, 92.26, 88.95, 90.25, 92.44 and 91.45; CP: 9.35, 13.38, 12.80, 18.8, 44.44 and 33.80; EE: 3.50, 3.39, 10.65, 2.50, 2.15 and 12.50; CF: 2.25, 9.92, 9.89, 16.0, 10.02 and 11.20; NFE: 82.37, 67.14, 54.72, 54.70, 37.97 and 35.00; and TA: 2.53, 6.17,11.94, 8.0, 5.42 and 7.50, respectively. The DM, CP, EE, CF, NFE and TA content (% DM basis) of concentrate mixture formulated, wheat straw and green berseem was 90.89, 20.25, 4.81, 9.35, 59.46 and 6.12; 89.35, 3.30, 1.10, 38.48, 47.24 and 9.88 and 20.00, 15.80, 1.40, 28.50, 38.30 and 16.00, respectively.

Table 01: Proximate composition of feed ingredients used in the experiment (% DM basis)

Feed ingredients	DM	CP	EE	CF	NFE	TA
Yellow maize	91.66	9.35	3.50	2.25	82.37	2.53
Wheat bran	92.26	13.38	3.39	9.92	67.14	6.17
Rice polish	88.95	12.80	10.65	9.89	54.72	11.94
Arhar chuni	90.25	18.8	2.50	16.0	54.70	8.0
Groundnut cake	92.44	44.44	2.15	10.02	37.97	5.42
Mustard cake	91.45	33.80	12.50	11.20	35.00	7.50
Concentrate mixture	90.89	20.25	4.81	9.35	59.46	6.12
Wheat straw	89.35	3.30	1.10	38.48	47.24	9.88
Berseem fodder	20.00	15.80	1.40	28.50	38.30	16.00

Table 02: Ingredients composition (%) of concentrate mixture

S. No.	Ingredients	Parts (%)
1	Maize	21.62
2	Groundnut cake	13.93
3	Mustard cake	13.63
4	Wheat bran	20.32
5	Rice polish	08.25
6	Arhar chuni	19.45
7	DCP	0.90
8	Mineral mixture	1.00
9	Common salt	0.90
	Total	100.00

Ingredient composition of the concentrate mixture fed to the experimental animals is given in Table 02.

4.2 Effects of supplemental prill fat and protected choline on performance of Murrah buffaloes

Data regarding effects of supplemental prill fat and protected choline on performance of buffaloes in terms of dry matter intake, body weight, body condition score, milk yield and milk composition during 90 days experimental period is presented in Tables 03, 04, 05, 06, 07 and 08, respectively.

4.2.1 Dry matter intake (kg/d)

Mean values of average daily dry matter intake of control, T_1 and T_2 groups at different fortnights are depicted in Table 03 and Figure 01. Analysis of variance revealed non-significant effect of supplementation of either prill fat alone (T_1) or protected choline along with prill fat (T_2) on dry matter intake of buffaloes. Average daily dry matter intake (kg/d) for control group at 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th} and 6^{th} fortnight was recorded as 13.32±0.45, 13.81±0.44, 14.18±0.34, 14.62±0.61, 14.99±0.66 and 15.02±0.72,

respectively, while that of T_1 and T_2 groups for corresponding fortnights was 13.44±0.40 and 13.61±0.42, 14.42±0.40 and 14.96±0.42, 14.92±0.88 and 15.48±0.95, 15.54±0.60 and 16.11±0.83, 15.95±0.70 and 16.48±0.81, and 16.00±0.94 and 16.65±0.85. Overall dry matter intake (kg/d) for control, T_1 and T_2 groups was recorded as 14.32±0.38, 15.04±0.45 and 15.54±0.54, respectively. The values for overall DMI (kg)/100 kg BW for control, T_1 and T_2 group were 2.81±0.12, 2.93±0.17 and 2.98±0.11, respectively while the respective overall DMI (kg)/kg $W^{0.75}$ was recorded as 0.130±0.00, 0.140±0.01 and 0.140±0.00.

Table 03: Mean dry matter intake (kg/d) for different group at fortnightly interval

Fortnights	Control	T_1	T_2	SEM	P Value
1	13.32±0.45	13.44±0.40	13.61±0.42	0.23	0.89
2	13.81±0.44	14.42±0.40	14.96±0.42	0.25	0.19
3	14.18±0.34	14.92±0.88	15.48±0.95	0.44	0.50
4	14.62±0.61	15.54±0.60	16.11±0.83	0.40	0.33
5	14.99±0.66	15.95±0.70	16.48±0.81	0.42	0.37
6	15.02±0.72	16.00±0.94	16.65±0.85	0.49	0.41
Overall DMI	14.32±0.38	15.04±0.45	15.54±0.54	0.28	0.20
Overall DMI (kg)/100 kg BW	2.81±0.12	2.93±0.17	2.98±0.11	0.08	0.66
Overall DMI (kg) /kg $W^{0.75}$	0.13±0.00	0.14±0.01	0.14±0.00	0.00	0.36

4.2.2 Body weight

Mean body weights recorded for control, T_1 and T_2 groups at 0 day (initial) and 90^{th} day (final) are presented in Table 04 and Figure 02. Analysis of variance revealed non-significant effect of supplementation of either prill fat alone (T_1) or protected choline along with prill fat (T_2) on average body weights of buffaloes at day 0 and 90^{th} day when compared to control group. Average body weights (kg) at the start of experiment i.e. on 0 day and at the end of feeding trial i.e. 90^{th} day for control, T_1 and T_2 groups

were recorded as 521.00±14.34 and 504.17±13.12, 524.00±23.28 and 513.83±23.91, and 526.50±12.72 and 518.17±13.51, respectively.

Table 04: Effect of supplemental prill fat and protected choline on mean body weight (kg/d) of Murrah buffaloes

Period	Control	T_1	T_2	SEM	P value
0 Day (Initial)	521.00±14.34	524.00±23.28	526.50±12.72	9.46	0.98
90^{th} Day (Final)	504.17±13.12	513.83±23.91	518.17±13.51	9.64	0.85

4.2.3 Body condition score (BCS)

Observations regarding mean body condition score (BCS) recorded for control group, prill fat group (T_1) and prill fat & choline group (T_2) at day 0 and 90^{th} day are depicted in Table 05 and 03. Analysis of variance revealed significant ($p<0.01$) effect of both the periods and treatments on BCS of buffaloes. Mean BCS recorded on 90^{th} day was significantly lower than the 0 day in all the three groups. Mean BCS of T_1 and T_2 group was significantly higher ($p<0.01$) than that of control group. The mean body condition score at 0 and 90^{th} day for control, T_1 and T_2 group was 3.05±0.02 and 2.49±0.03, 3.04±0.02 and 2.74±0.03, and 3.04±0.01 and 2.77±0.03, respectively.

Table 05: Effect of supplemental prill fat and protected choline on mean body condition score (BCS) of Murrah buffaloes

Groups	Periods		Treatment Mean ±SE	P value		
	0 Day	90^{th} Day		T	P	T*P
Control	3.05^{Aa}±0.02	2.49^{Bb}±0.03	2.77^{B}±0.08	<0.01	<0.01	<0.01
T_1	3.04^{Aa}±0.02	2.74^{Ab}±0.03	2.89^{A}±0.05			
T_2	3.04^{Aa}±0.01	2.77^{Ab}±0.03	2.90^{A}±0.04			

^{A,B} Means having different superscripts in the same column differ significantly at p<0.01
^{a,b} Means having different superscripts in the same row differ significantly at p<0.01

4.2.4 Lactation performance

4.2.4.1 Milk yield

The analysis of variance revealed significant effect of prill fat and protected choline on average milk yield (kg/d) in Murrah buffaloes. The average milk yield (kg/d) in buffaloes under different groups (Control, T_1 and T_2) is presented in Table 06 and Figure 04.

Table 06: Average milk yield (kg/d) at fortnightly interval in different groups of Murrah buffaloes

Fortnights	Control	T_1	T_2	Period Mean ± SE	P Value		
					T	P	T*P
Initial	6.31^{Da}±0.24	6.61^{Ca}±0.43	6.79^{Ca}±0.35	6.57^{E}±0.19	<0.01	<0.01	0.35
1	7.08^{Cc}±0.26	8.13^{Bb}±0.49	9.21^{Ba}±0.16	8.14^{D}±0.28			
2	7.79^{Bc}±0.24	8.76^{Bb}±0.20	9.83^{Ba}±0.17	8.79^{C}±0.23			
3	8.66^{Ac}±0.44	9.71^{Ab}±0.24	10.62^{Aa}±0.09	9.67^{B}±0.25			
4	9.29^{Ac}±0.30	10.45^{Ab}±0.14	11.21^{Aa}±0.11	10.32^{A}±0.22			
5	9.14^{Ac}±0.18	10.33^{Ab}±0.30	11.11^{Aa}±0.08	10.19^{A}±0.22			
6	9.01^{Ac}±0.18	10.18^{Ab}±0.26	10.95^{Aa}±0.15	10.05^{AB}±0.22			
Overall	8.18^{c}±0.21	9.17^{b}±0.27	9.96^{a}±0.05				

^{A,B,C,D,E} Means having different superscripts in the same column differ significantly at p< 0.01

^{a,b,c} Means having different superscripts in the same row differ significantly at p<0.01

The average milk yield (kg/d) in control group ranged from 6.31±0.24 to 9.29±0.30, while that of T_1 and T_2 groups ranged from 6.61±0.43 to 10.45±0.14 and 6.79±0.35 to 11.21±0.11, respectively. The average milk yield of control group at day 0 day, 1st, 2nd, 3rd, 4th, 5th and 6th fortnight was recorded as 6.31±0.24, 7.08±0.26, 7.79±0.24, 8.66 ±0.44, 9.29 ±0.30, 9.14±0.18 and 9.01±0.18, respectively; the corresponding values for T_1 and T_2 at 0 day, 1st, 2nd, 3rd, 4th, 5th and 6th fortnight were 6.61±0.43 and 6.79±0.35, 8.13±0.49 and 9.21±0.16, 8.76±0.20 and 9.83±0.17, 9.71±0.24 and 10.62±0.09, 10.45±0.14 and 11.21±0.11, 10.33±0.30 and 11.11±0.08 and 10.18±0.26 and 10.95±0.15. At 0 day there was no any significant difference among milk yield in different groups i.e. control, T_1 and T_2, but average milk

yield (kg/d) recorded at fortnightly interval up to 6th fortnight differed significantly ($p<0.01$). The overall average milk yield (kg/d) and average milk yield (kg/d) at every fortnight in T_2 group was recorded to be significantly ($p<0.01$) highest followed by T_1 and control group. Overall average milk yield (kg/d) of T_1 and T_2 groups was 0.99 kg and 1.78 kg higher as compared to control group.

4.2.4.2 Fat corrected milk (FCM)

Data regarding average 4% fat corrected milk (FCM) for control, T_1 and T_2 groups at 30th, 60th and 90th day is presented in Table 07. Analysis of variance revealed significant ($p<0.01$) effect of various treatments on FCM (kg/day) during different periods. Mean FCM (kg/d) was found to be significantly ($p<0.01$) highest for T_2 followed by T_1 and control group. Within same treatment group highest mean FCM was recorded at 90th day followed by 60th and 30th day. Average FCM (kg/d) for control group at 30th, 60th and 90th day was recorded as 9.87±0.31, 12.29±0.43 and 12.80±0.26, respectively, the corresponding values for T_1 and T_2 groups were 11.47±0.46 and 13.23±0.22, 14.43±0.20 and 16.18±0.12, and 15.38±0.38 and 17.25±0.26. Mean FCM (kg/d) of T_1 and T_2 groups was 2.11 and 3.90 kg higher than control group.

4.2.4.3 Energy corrected milk (ECM)

Data regarding average energy corrected milk (ECM) for control, T_1 and T_2 groups at different periods i.e. 30th, 60th and 90th day is presented in Table 07. Analysis of variance employed to find out the effect of periods and treatment revealed significant ($p<0.01$) effect of period and treatment on ECM (kg/d) of in buffaloes. The significantly ($p<0.01$) highest mean ECM (kg/d) was recorded in T_2 group followed by T_1 and control. The ECM values at 60th and 90th day did not differ significantly among control and treatment groups. The average values of ECM (kg/d) recorded at 30th, 60th and 90th day for control, T_1 and T_2 groups were 10.14±0.29, 12.59±0.41 and 13.13±0.27; 11.75±0.44, 14.89±0.23 and 15.64±0.40 and 13.55±0.24, 16.55±0.18 and 17.41±0.24, respectively.

4.2.4.4 Fat yield

Mean values of average fat yield (kg/day) for control and treatment groups at different period i.e. 30th, 60th and 90th day are as presented in Table 07. Mean fat yield (kg/d) was significantly (p<0.01) highest in T_2 (0.76±0.02) followed by T_1 (0.66±0.02) and control (0.55±0.02) group. Mean fat yield (kg/d) in control group at 60th and 90th day was significantly (p<0.01) higher than that at 30th day, whereas fat yield of T_1 and T_2 group differed significantly (p<0.01) at each interval i.e. 30th, 60th and 90th day. Average fat yield (kg/d) of control, T_1 and T_2 groups at 30th, 60th and 90th was 0.46±0.02, 0.58±0.02 and 0.61±0.01; 0.54±0.02, 0.69±0.01 and 0.75±0.02, and 0.63±0.01, 0.79±0.01 and 0.86±0.01, respectively.

Table 07: Effect of supplemental prill fat and protected choline on lactation performance of Murrah buffaloes

Attributes	Periods			Treatment Mean± SE	P Value		
	30th Day	60th Day	90th Day		T	P	T*P
4% FCM (kg/d)							
Control	9.87^{Cb}±0.31	12.29^{Ca}±0.43	12.80^{Ca}±0.26	11.65^{C}±0.36	<0.01	<0.01	0.46
T_1	11.47^{Bc}±0.46	14.43^{Bb}±0.20	15.38^{Ba}±0.38	13.76^{B}±0.45			
T_2	13.23^{Ac}±0.22	16.18^{Ab}±0.12	17.25^{Aa}±0.26	15.55^{A}±0.43			
ECM (kg/d)							
Control	10.14^{Cb}±0.29	12.59^{Ca}±0.41	13.13^{Ca}±0.27	11.95^{C}±0.36	<0.01	<0.01	0.58
T_1	11.75^{Bb}±0.44	14.89^{Ba}±0.23	15.64^{Ba}±0.40	14.09^{B}±0.45			
T_2	13.55^{Ab}±0.24	16.55^{Aa}±0.18	17.41^{Aa}±0.24	15.84^{A}±0.42			
Fat Yield (kg/d)							
Control	0.46^{Cb}±0.02	0.58^{Ca}±0.02	0.61^{Ca}±0.01	0.55^{C}±0.02	<0.01	<0.01	0.18
T_1	0.54^{Bc}±0.02	0.69^{Bb}±0.01	0.75^{Ba}±0.02	0.66^{B}±0.02			
T_2	0.63^{Ac}±0.01	0.79^{Ab}±0.01	0.86^{Aa}±0.01	0.76^{A}±0.02			

^{A,B,C} Means having different superscripts under particular attribute in the same column differ significantly at p<0.01

^{a,b,c} Means having different superscripts in the same row differ significantly at p<0.01

4.2.5 Milk composition

4.2.5.1 Milk fat

Data for average milk fat (%) recorded for control, T_1 and a T_2 group is depicted in Table 08 and Figure 05. Analysis of variance employed

for determining the effect of period and treatment revealed significant ($p<0.01$) effect of both on milk fat (%). The overall mean milk fat (%) was significantly ($p<0.01$) highest in T_2 group (7.19±0.12) followed by T_1 (6.87±0.12) and control (6.46±0.06). The value for average milk fat (%) for control, T_1 and T_2 group at 30th, 60th and 90th days were 6.19±0.03, 6.47±0.05 and 6.74±0.05; 6.40±0.17, 6.88±0.09 and 7.34±0.12 and 6.60±0.09, 7.21±0.09 and 7.76±0.09, respectively. Average milk fat (%) of T_1 and T_2 groups was increased by 0.41 and 0.73 units than control group.

Table 08: Effect of supplemental prill fat and protected choline on milk composition of Murrah buffaloes

Attributes	Periods			Treatment Mean± SE	P Value		
	30th Day	60th Day	90th Day		T	P	T*P
Fat (%)							
Control	6.19^{Bc}±0.03	6.47^{Cb}±0.05	6.74^{Ca}±0.05	6.46^{C}±0.06			
T_1	6.40^{ABc}±0.17	6.88^{Bb}±0.09	7.34^{Ba}±0.12	6.87^{B}±0.12	<0.01	<0.01	0.04
T_2	6.60^{Ac}±0.09	7.21^{Ab}±0.09	7.76^{Aa}±0.09	7.19^{A}±0.12			
SNF (%)							
Control	9.23±0.07	9.68±0.15	9.68±0.18	9.53±0.09			
T_1	9.56±0.18	10.03±0.19	9.63±0.23	9.74±0.12	0.19	0.14	0.08
T_2	9.97±0.14	9.80±0.20	9.52±0.11	9.77±0.10			
Total Solid (%)							
Control	15.42^{Cb}±0.08	16.15^{Ca}±0.16	16.42^{Ca}±0.19	15.99^{C}±0.13			
T_1	15.96^{Bb}±0.29	16.91^{Ba}±0.20	16.97^{Ba}±0.15	16.61^{B}±0.16	<0.01	<0.01	0.75
T_2	16.57^{Ab}±0.20	17.02^{Aa}±0.24	17.28^{Aa}±0.12	16.96^{A}±0.13			
Protein (%)							
Control	3.30±0.12	3.34±0.14	3.43±0.11	3.36±0.07			
T_1	3.31±0.14	3.60±0.10	3.46±0.09	3.46±0.07	0.45	0.13	0.69
T_2	3.36±0.06	3.54±0.09	3.42±0.04	3.44±0.04			
Lactose (%)							
Control	4.77±0.17	5.01±0.20	5.05±0.17	4.94±0.10			
T_1	4.97±0.21	5.39±0.15	5.15±0.16	5.17±0.10	0.33	0.09	0.94
T_2	4.89±0.34	5.18±0.06	5.20±0.06	5.09±0.11			
Density (g/cm³)							
Control	1.033±0.001	1.033±0.001	1.032±0.001	1.033±0.001			
T_1	1.033±0.001	1.032±0.001	1.031±0.001	1.032±0.001	0.56	0.12	0.92
T_2	1.033±0.001	1.031±0.001	1.031±0.002	1.032±0.001			

^{A,B,C} Means having different superscripts under particular attribute in the same column differ significantly at p<0.01

^{a,b,c} Means having different superscripts in the same row differ significantly at p<0.01

4.2.5.2 Solid not fat (SNF)

Data for average milk SNF (%) recorded for control, T_1 and T_2 groups is depicted in Table 08 and Figure 06. Analysis of variance revealed non-significant effect of treatment and period on milk SNF (%). At 30th day numerically highest milk SNF (%) was recorded in T_2 (9.97±0.14) followed by T_1 (9.56±0.18) and control (9.23±0.07) group. At 60th day the numerically highest value of milk SNF (%) was recorded for T_1 (10.03±0.19) followed by T_2 (9.80±0.20) and control (9.68±0.15) group. The values of mean milk SNF (%) on 90th day was numerically higher in control group (9.68±0.18) followed by T_1 (9.63±0.23) and T_2 (9.52±0.11). The overall mean milk SNF (%) in control, T_1 and T_2 group was 9.53±0.09, 9.74±0.12 and 9.77±0.10, respectively.

4.2.5.3 Total solid

Observations regarding average milk total solid (%) of all the groups during different periods are presented in Table 08 and 07. Analysis of variance employed for knowing the effect of period and treatment revealed significant effect ($p<0.01$) on milk total solid (%). The average milk total solid (%) of all the three groups was significantly ($p<0.01$) increased at 60th and 90th day as compared to that at 30th day. The overall mean of milk total solid (%) was significantly ($p<0.01$) higher in T_2 (16.96±0.13) followed by T_1 (16.61±0.16) and control (15.99±0.13) group. Milk total solid (%) showed increasing trend over the period. The highest milk total solid (%) for each group was recorded at 90th day with the values being 16.42 ±0.19, 16.97 ±0.15 and 17.28 ±0.12 for control, T_1 and T_2 groups, respectively.

4.2.5.4 Protein

Data regarding milk protein (%) for control, T_1 and T_2 groups at 30th, 60th and 90th day is presented in Table 08 and Figure 08. Analysis of variance employed to find out the effect of period and treatment revealed non-significant effect on milk protein (%). The mean milk protein (%) of control, T_1 and T_2 groups at 30th, 60th and 90th day was 3.30±0.12, 3.34±0.14 and 3.43±0.11; 3.31±0.14, 3.60±0.10 and 3.46±0.09 and 3.36±0.06, 3.54±0.09 and 3.42±0.04, respectively. The overall mean milk protein (%) for control, T_1 and T_2 group was recorded as 3.36±0.07, 3.46±0.07 and 3.44±0.04, respectively.

4.2.5.5 Lactose

Observations regarding average milk lactose (%) recorded for control and treatment groups at different periods are depicted in Table 08 and Figure 09. Analysis of variance conducted to find out the effect of period and treatment revealed non-significant effect on milk lactose (%). The mean milk lactose (%) of control, T_1 and T_2 groups at 30^{th}, 60^{th} and 90^{th} day was 4.77±0.17, 5.01±0.20 and 5.05±0.17; 4.97±0.21, 5.39±0.15 and 5.15±0.16 and 4.89±0.34, 5.18±0.06 and 5.20±0.06, respectively. The overall mean milk lactose (%) for control, T_1 and T_2 group was 4.94±0.10, 5.17±0.10 and 5.09±0.11, respectively.

4.2.5.6 Density

Observations regarding milk density (g/cm^3) recorded for control and treatment groups at different periods are depicted in Table 08 and Figure 10. Analysis of variance revealed non-significant effect of period and treatment on milk density (g/cm^3). The mean milk density (g/cm^3) for control, T_1 and T_2 groups at 30^{th}, 60^{th} and 90^{th} was 1.033±0.001, 1.033±0.001 and 1.032±0.001; 1.033±0.001, 1.032±0.001 and 1.031±0.001 and 1.033±0.001, 1.031±0.001 and 1.031±0.002, respectively. The overall mean milk density (g/cm^3) for control, T_1 and T_2 group was 1.033±0.001, 1.032±0.001 and 1.032±0.001, respectively

4.3 Effects of supplemental prill fat and protected choline on blood metabolic profile of Murrah buffaloes

4.3.1 Glucose

The mean blood glucose levels (mg/dl) of all experimental buffaloes recorded at 0^{th} and 90^{th} day are presented in Table 09 Figure 11. Analysis of variance revealed significant ($p<0.01$) effect of period on glucose level while the effect of treatment was non-significant. The overall mean glucose level for control, T_1 and T_2 group was found to be non-significant. The mean glucose (mg/dl) level of buffaloes from control, T_1 and T_2 group at 0 day was 51.33±0.88, 51.83±0.95 and 52.00±1.29, corresponding values at 90^{th} day were 52.67±1.05, 54.67±1.05 and 55.33±1.28. The overall mean glucose level (mg/dl) for control, T_l and T_2 group was found to be 52.00±0.69, 53.25±0.80 and 53.67±1.00, respectively.

4.3.2 Cholesterol

The mean blood cholesterol (mg/dl) levels of all experimental buffaloes in control and treatment groups are presented in Table 09 Figure 11. Analysis of variance revealed non-significant effect of treatment on blood cholesterol level (mg/dl) while the effect of period was found to be significant ($p<0.01$). The blood cholesterol level (mg/dl) at 0 day for control, T_1 and T_2 groups was 127.98±2.23, 128.13±2.33 and 128.19±1.38, respectively, while corresponding values at 90th day were 167.32±3.41, 166.12±2.42 and 165.40±2.62 for control, T_1 and T_2 group, respectively. The overall mean blood cholesterol levels (mg/dl) in control, T_1 and T_2 groups were 147.65±6.24, 147.13±5.95 and 146.79±5.78, respectively.

4.3.3 Triglyceride

The mean blood triglyceride levels (mg/dl) of all experimental buffaloes at 0 and 90th day in control, T_1 and T_2 group are shown in Table 09 and Figure 11. Analysis of variance employed for finding the effect of treatments and periods revealed significant ($p<0.01$) effect of both on blood triglyceride levels (mg/dl). Significant difference ($p<0.01$) was observed in blood triglyceride levels (mg/dl) between control and treatment groups on 90th day while on 0 day, it was found to be non-significant. The Mean blood triglyceride levels (mg/dl) of buffaloes in control, T_1 and T_2 group at 0 and 90th day were 23.90±0.80 and 25.07±0.51; 24.06±1.00 and 30.30±0.90 and 24.02±1.21 and 32.51±0.82, respectively. The overall mean blood triglyceride (mg/dl) levels of T_1 (27.18±1.14) and T_2 (28.26±1.46) group were 10.98 and 15.39% higher, as compared to control (24.49±0.49) group.

4.3.4 NEFA

The mean blood NEFA concentrations (mmol/l) of all experimental buffaloes in control and treatment groups at 0 and 90th day are presented in Table 09 and Figure 12. Analysis of variance revealed significant ($p<0.01$) effect of period and treatment on blood NEFA concentrations (mmol/l). Blood NEFA concentrations (mmol/l) was increased significantly ($p<0.01$) at 90th day in control group whereas, the same was reduced significantly ($p<0.01$) in T_1 and T_2 groups at 90th day as compared to 0 day.

Mean values of blood NEFA concentration (mmol/l) for control, T_1 and T_2 group at 0 and 90th day were observed to be 0.46±0.01 and 0.54±0.01, 0.47±0.01 and 0.44±0.01 and 0.44±0.02; and 0.38±0.01, respectively. The overall mean NEFA concentration (mmol/l) was significantly (p<0.01) higher in control group (0.50±0.01) followed by T_1 (0.45±0.01) and T_2 (0.41±0.01). The overall mean NEFA concentration (mmol/l) of T_1 and T_2 group was 10.0 and 18.0% lower than control group.

Table 09: Effect of supplemental prill fat and protected choline on blood biochemical profile of Murrah buffaloes

Attributes	Periods		Treatment Mean± SE	P Value		
	0 Day	90th Day		T	P	T*P
Glucose (mg/dl)						
Control	51.33^{Ab}±0.88	52.67^{Aa}±1.05	52.00^{A}±0.69	0.30	0.01	0.64
T_1	51.83^{Ab}±0.95	54.67^{Aa}±1.05	53.25^{A}±0.80			
T_2	52.00^{Ab}±1.29	55.33^{Aa}±1.28	53.67^{A}±1.00			
Cholesterol (mg/dl)						
Control	127.98^{Ab}±2.23	167.32^{Aa}±3.41	147.65^{A}±6.24	0.94	<0.01	0.91
T_1	128.13^{Ab}±2.33	166.12^{Aa}±2.42	147.13^{A}±5.95			
T_2	128.19^{Ab}±1.38	165.40^{Aa}±2.62	146.79^{A}±5.78			
Triglyceride (mg/dl)						
Control	23.90^{Ab}±0.80	25.07^{Ba}±0.51	24.49^{B}±0.49	<0.01	<0.01	<0.01
T_1	24.06^{Ab}±1.00	30.30^{Aa}±0.90	27.18^{A}±1.14			
T_2	24.02^{Ab}±1.21	32.51^{Aa}±0.82	28.26^{A}±1.46			
NEFA (mmol/l)						
Control	0.46^{Ab}±0.01	0.54^{Aa}±0.01	0.50^{A}±0.01	<0.01	0.88	<0.01
T_1	0.47^{Aa}±0.01	0.44^{Bb}±0.01	0.45^{B}±0.01			
T_2	0.44^{Aa}±0.02	0.38^{Cb}±0.01	0.41^{C}±0.01			

^{A,B,C} Means having different superscripts under particular attribute in the same column differ significantly at p<0.01

^{a,b} Means having different superscripts in the same row differ significantly at p<0.01

4.4 Effects of supplemental prill fat and protected choline on economics of production in Murrah buffaloes

Cost of feeding concentrate mixture, dry roughage, green berseem and supplemental prill fat along with protected choline chloride for control, T_1 and T_2 groups is presented in Table 10 and Figure 13. Average total feed cost/animal/day (Rs.) for control, T_1 and T_2 group was calculated to be 139.86, 177.27 and 201.11, respectively. Cost of feed/kg milk produced/per animal/day (Rs.) was observed to be Rs 17.09, 19.33 and 20.19 for control, T_1 and T_2 group, respectively. Highest net profit/animal/day (Rs.) was observed in T_2 group, however it was 8.25 and 3.12% higher in T_2 (247.09) and T_1 (235.38) than control (228.24) group.

Table 10: Economics of prill fat and protected choline supplemented Murrah buffaloes

Attributes	Control	T_1	T_2
Cost of Concentrate/ Animal/ Day (Rs.)	77.71	82.48	87.59
Cost of Dry Roughage/ Animal/ Day (Rs.)	30.15	30.54	30.87
Cost of Green Roughage / Animal/ Day (Rs.)	32.00	30.50	31.00
Cost of Prill Supplementation / Animal/ Day (Rs.)	Nil	33.75	34.81
Cost of Choline Chloride/ Animal/ Day (Rs.)	Nil	Nil	16.84
Total Feed Cost/ Animal/ Day (Rs.)	139.86	177.27	201.11
Average Milk Yield/ Animal/ Day (kg)	08.18	09.17	09.96
Return from Milk Produced/ Animal/ Day (Rs.)	368.10	412.65	448.20
Cost of Feed/kg Milk Produced/ Animal/ Day (Rs.)	17.09	19.33	20.19
Net Profit/ Animal/ Day (Rs.)	228.24	235.38	247.09

*****Feeds cost (Rs./kg); Concentrate:17.99, Dry Roughage: 3.47, Green Roughage: 2.00, Prill Fat: 90.00, Choline: 312.00; Milk Cost (Rs./kg): 45.00

5. DISCUSSION

Results obtained in context of effect of prill fat and protected choline supplementation on performance, blood metabolic profile and economics of production of lactating Murrah buffaloes in the present experiment is discussed in this chapter under various heads:

5.1 Proximate composition of feed ingredients

Chemical composition of feed ingredients used in preparing experimental diets indicated that there was not much variation in their chemical composition when compared with the values reported by other workers (Ranjhan, 1998; Baghel and Netke, 1992 and Khare, 2013).

5.2 Effects of supplemental prill fat and protected choline on performance of Murrah buffaloes

5.2.1 Dry matter intake

In present study treatment means of the average daily dry matter intake, dry matter intake per 100 kg body weight and dry matter intake per kg metabolic body size indicated that supplementation of basal diet with either prill fat or prill fat + protected choline in buffaloes did not influenced ($p>0.01$) their dry matter intake as compared to control group. However, dry matter intake (DMI) was numerically higher for treatment groups compared to control group. Some workers too have reported that daily dry matter intake of animals is not affected by supplementation of rumen bypass fat (Tyagi *et al.,* 2009 and Sirohi *et al*., 2010).

Findings of present study regarding non-significant change in dry matter intake in buffaloes is supported by studies conducted by Garg *et al.* (2012b) and Garg *et al.* (2012a) where they also did not reported any significant change in dry matter intake of Jaffarabadi buffaloes and crossbred cows after supplementation of either prill fat or prill fat + choline when compared to control group fed basal ration, however levels of prill fat (100 g/d in cow; 150 g/d in buffaloes) and protected choline (10 g/d for cow; 15 g/d for

buffalo) used by them were quite lower than the levels used in present study. In support to our results, Rajesh *et al*. (2014) also reported that prill fat supplementation augmented milk secretion of cows without affecting dry matter intake significantly.

The non-significant effect of prill fat on DMI in supplemented group is in line with earlier findings of various researchers in cows during early and mid-lactation (Grummer, 1993; Theurer *et al*., 2009; Stusinka *et al.,* 2006; Thakur and Shelke., 2010; Silvestre *et al.,* 2011 and Singh *et al.,* 2014).

5.2.2 Body weight

In the present study treatment means of the average body weight (kg) indicated that supplementation of basal diet with either prill fat or prill fat + protected choline in buffaloes did not influenced ($p>0.01$) their body weight as compared to control group. However, losses in body weights of buffaloes under control group were higher (3.23%) during the study but recoveries of body weight losses were better (1.94 and 1.58%) in supplemental T_1 and T_2 groups indicating thereby that some of the additional energy intake by cows fed prill fat supplemented diets may have contributed to preventing BW loss, which is often seen during early lactation (Ganjkhanlou *et al*., 2009).

Lipid mobilized from body reserves makes a substantial contribution to the energetic cost of milk production in early lactation (Friggens *et al*., 2004). Effect of supplemental Ca-LCFA (Calcium coated long chain fatty acids) on change of BW is influenced by parity of animal; loss of BW is more and longer lasting in primiparous cows than multiparous cows (Sklan *et al.*, 1994). In support to our results, non significant effect of supplemental Ca-LCFA was reported by Tyagi *et al.* (2009) on changes of BW of dairy cows however, recovery of BW losses was better in Ca-LCFA supplemented cows in early lactation. Observations regarding non significant effect of protected choline on body weight of animals are in accordance with Zom *et al*. (2011) who reported no change in body weight of peripatuirent cows after supplementing protected choline in their diet.

5.2.3 Body condition score

The addition of prill fat alone or prill fat + protected choline in the diets of experimental buffaloes did influence their body condition score statistically ($p<0.01$) and was better when compared with control.

Buffaloes fed basal ration without any supplementation showed a greater loss of body condition than supplemented animals, which is in accordance with their reduced energy balance. These results regarding improvement in BCS after prill fat supplementation are supported by earlier workers (Rajesh *et al*., 2014 and Yadav *et al*., 2015). Lima *et al*. (2007) also reported improvement in BCS of experimental cows after supplementation of RPC @ 15 g/d.

Contrary to our results, Ganjkhanlou *et al*. (2009) reported no change in body condition scores of Holstein cows after addition of rumen protected fat to their diets during early lactation. Naik *et al.* (2009) also reported no significant effect of supplemental bypass fat on BCS of dairy cows; however, recovery of BCS losses was better in bypass fat supplemented cows during early lactation.

5.2.4 Milk yield (kg/d)

In the present study treatment means of the average milk yield (kg/d) and fat corrected milk yield (kg/d) indicated that supplementation of basal diet with either prill fat alone or along with protected choline in the diets of buffaloes significantly ($p<0.01$) improved their milk yield and fat corrected milk yield as compared to control group. Overall average milk yield (kg/d) was increased by 12.10 and 21.76% in prill fat alone and prill fat + protected choline supplemented groups over the control group. The higher milk productions in supplemented groups were attributed to more TDN intake in conjunction with prill fat which increased the energy density of ration and reduced deleterious effect of negative energy balance as evident from lower blood NEFA levels.

The significant increase in milk production in prill fat supplemental group is well corroborated with findings of many researchers

reporting an increased milk yield between 0.40-3.11 kg/d in experimental cows (Shelke *et al*., 2011; Fahey *et al.,* 2002; McNamara *et al.,* 2003; Mishra *et al*., 2004 and Salem and Bouraoui, 2008). Similarly, Kumar and Thakur (2007); Garg *et al*. (2008); Barley and Baghel (2009); Sirohi *et al.* (2010) and Rajesh *et al.* (2014) also reported significant improvement in milk yield of ruminants fed bypass fat. However, no improvement in milk yield in bypass fat supplemented cows have also been reported by some researchers which could be due to different degree of inertness and amount of dietary fat offered (Klusmeyer *et al*.,1991; Sklan *et al*., 1992 and Elliott *et al.,* 1996).

Elek *et al.* (2008) and Lima *et al.* (2007) observed significant improvement in milk yield of dairy cows after supplementing rumen protected choline (RPC) to basal diet. Pinotti *et al*. (2002) reported that RPC may improve the milk yield of dairy animals by elevating the export of triglycerides from the liver and by sparing methionine as a methyl donor. Collectively, the study indicated that further improvements in milk production in response to RPC supplementation may be attributed to a methyl donor sparing effect. Thus, enhanced intestinal supply of choline might have further improved milk production in these Murrah buffaloes.

5.2.5 Milk composition

In present study milk fat (%) and TS (%) was significantly higher ($p<0.01$) in supplemented groups than the control. Milk lactose (%), SNF (%), density (g/cm^3) and protein (%) content were not influenced by feeding of prill fat alone or along with protected choline. In present study milk fat (%) was increased by 0.41 and 0.73 units in prill fat and prill fat + protected choline supplemented groups over the control group. Significant ($p<0.01$) increase in milk fat and total solid (TS) of prill fat supplemented group is supported by Mishra *et al.* (2004); Garg *et al.* (2008) and Sirohi *et al.* (2010). Lima *et al*. (2007) and Garg *et al.* (2012b) supports our results regarding significant higher milk fat (%) in RPC supplemented groups. Non significant change in milk protein (%) of supplemented groups is supported by Sirohi *et al*. (2010). In contrary to our results, Polidori *et al*. (1997) has reported decreased milk protein level in experimental buffaloes after bypass fat supplementation.

The study has made it amply clear that medium and high producing lactating buffaloes do need the bypass fat supplement in their diet, in order to meet their energy requirements fully to express their milk production potential. This was demonstrated by the highly significant increase in milk yield, FCM yield, fat percentage and TS percentage in milk as a result of feeding the prill fat alone or along with protected choline.

The increase in milk fat content in prill fat or prill fat + protected choline supplemented group may be due to availability of more fatty acid (SFA and USFA) to the mammary gland and their incorporation into milk fat (Gulati *et al*., 2003). Further, as choline is used for phospholipid synthesis its supplementation facilitates lipid absorption and transport, thereby favouring milk fat synthesis.

5.3 Effects of supplemental prill fat and protected choline on blood metabolic profile of Murrah buffaloes

Blood glucose (mg/dl) level was not influenced by feeding prill fat alone or along with protected choline. The reason may be a high metabolic rate of utilization of glucose and homeostatic mechanism of animal body does not allow appreciable change in glucose level. Similar results regarding non significant change in blood glucose levels are reported by Fahey *et al*. (2002) and Tyagi *et al*. (2009) on supplementing Ca salts of fatty acids and bypass fat in experimental animals. The non-significantly higher glucose level might have contributed in enhancement of milk yield as glucose is the main precursor for lactose synthesis.

Supplementation of either prill fat alone or along with protected choline did not show any significant effect on blood cholesterol levels (mg/dl) of experimental buffaloes. Cholesterol is a component of the serum lipoproteins and its concentration in serum gives an indication of overall lipoprotein concentrations. Results obtained in present study are in agreement with the previous findings of Pinotti *et al*. (2004) and Janovick *et al*. (2006). In contrary, Zahra *et al*. (2006) observed reduced level of cholesterol on supplementation of RPC to dairy cows.

In present study in blood triglyceride (mg/dl) level was found to be increased significantly ($p<0.01$) by 10.98 and 15.39% in prill fat and prill fat + protected choline supplemented groups as compared to control group. In support to our results, Kumar and Thakur (2007) and Barley and Baghel (2009) have reported increased levels of triglycerides after supplementing bypass fat to buffaloes.

At the beginning of the lactation cycle, the blood NEFA originating from mobilization of adipose tissue is elevated, mainly due to a negative energy balance. Increased concentrations indicate lipolysis, which occurs in response to increased energy demand. Concentrations of NEFA in plasma can reflect the degree of adipose tissue mobilization, but might also be affected by the supply of fatty acids to the small intestine (Drackley, 1999). Blood concentration of NEFA reflects the balance between release of fatty acids from adipose tissue and lipoproteins, and utilization of NEFA by tissues, such as the mammary gland. Small changes in plasma NEFA are observed with fat supplementation, but in early lactation, most of the changes in NEFA concentrations are the result of changes in energy status and adipose tissue mobilization (Juchem *et al*., 2008).

In present study blood NEFA concentration (mmol/l) was decreased significantly ($p<0.01$) by 10.0 and 18.0% in prill fat and prill fat + protected choline supplemented groups as compared to control group. The lower NEFA level in supplemented group cows suggests beneficial effect of prill fat feeding in restricting the body reserve mobilization in early lactation of cows. Although an increase in mobilization on supplementation of bypass fat (Delbecchi *et al*., 2001) or decrease in body lipid mobilization during postpartum period have also been reported (Grummer, 1995; Grum *et al*., 1996). Results regarding significant reduction in serum NEFA level of RPC supplemented group are supported by Lima *et al*. (2007); Zahra *et al*. (2006); Garg *et al.* (2012a) and Garg *et al.* (2012b). In accordance to our results regarding significantly ($p<0.01$) lower blood NEFA concentration in prill fat supplemented buffaloes, Singh *et al.* (2014) and Yadav *et al.* (2015) have also reported reduced blood NEFA concentration in bypass fat supplemented animals.

5.4 Effects of supplemental prill fat and protected choline on economics of production in Murrah buffaloes

In present study net profit/animal/day (Rs.) was increased by 3.12 and 8.25% in prill fat and prill fat + protected choline supplemented group over the non-supplemented group which is attributed to higher milk yield in supplemented groups.

These findings are in consonance with the findings of Yadav *et al*. (2015) where they reported that the supplementation of prill fat in the ration of crossbreed cows was economical and resulted in an additional income generation of Rs. 94.46/cow/day. Results regarding higher net profit in bypass fat supplemented group are also in accordance with Naik *et al.* (2009); Gowda *et al*. (2013) and Singh *et al.* (2014). In Support to our results, Mohsen *et al*. (2011) also reported higher net profit in RPC supplemented animals.

6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 Summary

Present study was designed to evaluate the effect of prill fat and protected choline supplementation on performance of lactating Murrah buffaloes. Murrah buffaloes (*n*=18) yielding 8-10 kg milk/head/day were divided into three groups of six each, based on milk yield, fat per cent and stage of lactation. All buffaloes were fed similar basal diet, comprising 12-15 kg green fodder, 4-6 kg wheat straw and concentrate mixture. Concentrate mixture was given according to the level of milk production to meet the requirements for maintenance and milk production as per feeding standard given by Kearl (1982). Buffaloes in group T_1 were daily supplemented with prill fat @ 2.5% of total DMI per animal whereas, buffaloes in group T_2 were supplemented with 54 g rumen protected choline (RPC) along with prill fat fed @ 2.5% of total DMI per animal. Feeding trial was conducted for 90 days. Observations on daily feed intake, daily milk yield, fat per cent etc. were recorded during the experiment. Body weights and body condition score of each buffalo under different groups were recorded at the start and at completion of experiment. Milk samples were collected on 30th day, 60th day and 90th day from each buffalo in different treatment groups and were analyzed for its composition. Blood samples were collected from all the experimental buffaloes at 0 day and at the end of experiment to estimate various blood biochemical parameters including NEFA. Economics of milk production over feed cost was also calculated in different treatment groups.

Average daily dry matter intake (kg/d) of buffaloes was not affected by supplementation of either prill fat alone or along with rumen protected choline chloride in their basal ration, Overall DMI (kg/d) and DMI (kg) per 100 kg body weight or per kg metabolic body size in prill fat and prill fat along with rumen protected choline chloride supplemented buffaloes were similar to control group. Similarly, body weights (Kg) of experimental buffaloes were not affected by supplementation of either prill fat alone or along with rumen protected choline chloride in their basal ration.

The overall BCS during postpartum period in prill fat and prill fat along with rumen protected choline chloride supplemented groups was significantly ($p<0.01$) higher than control group (2.77±0.08 vs. 2.89±0.05; 2.90 ±0.04). Decline in BCS was reported more in control group than prill fat and prill fat along with rumen protected choline chloride supplemented group buffaloes during early lactation.

Average milk yield (kg/d) and fat (%) in prill fat and prill fat along with rumen protected choline chloride supplemented groups were significantly ($p<0.01$) increased by 0.99 kg and 0.41 units and 1.78 kg and 0.73 units, respectively as compared to control group, similarly, average FCM of respective treatment groups was 2.11 kg and 3.90 kg higher than that of control group. Further, percent increase in energy corrected milk (kg/d) and fat yield (kg/d) in prill fat and prill fat along with rumen protected choline chloride supplemented groups was 17.90 and 20.00% and 32.55 and 38.18%, respectively when compared to control group.

Milk total solids content was improved significantly ($p<0.01$) in prill fat and prill fat along with rumen protected choline chloride supplemented groups, whereas milk protein, solids-not-fat, lactose and density remained unaffected in supplemented groups as compare to control group.

Non-esterified fatty acids levels in the blood serum were decreased by 10.0 and 18.0% ($p<0.01$) in prill fat and prill fat along with rumen protected choline chloride supplemented groups, as compared to control group. There was significant ($p<0.01$) improvement in triglycerides levels in the blood serum of both the groups supplemented with either prill fat alone or along with rumen protected choline chloride as compared to control, the overall mean blood triglyceride (mg/dl) levels of respective supplemented groups were 10.98 and 15.39% higher, as compared to control group. However, supplementation of prill fat either alone or along with rumen protected choline chloride had no influence ($p>0.05$) on glucose and cholesterol levels in the blood serum of experimental buffaloes.

Net profit/animal/day (Rs.) was 3.12 and 8.25% higher in prill fat and prill fat along with rumen protected choline chloride supplemented groups than control.

6.2 Conclusions

- The supplementation of prill fat and prill fat along with rumen protected choline chloride did not affect feed intake.
- Supplementation of prill fat (2.5% of DMI) and prill fat along with rumen protected choline chloride (54g/animal/d) in early lactating Murrah buffaloes significantly increased the milk yield and milk fat up to 12.10 and 21.76% and 6.34 and 11.3%, respectively, over the control group.
- Blood triglyceride (mg/dl) level was significantly ($p<0.01$) increased whereas NEFA (mmol/l) level was significantly reduced in protected choline + prill fat supplemented group followed by prill fat supplemented group as compare to control group. Other blood metabolites were not affected due to supplementation.
- Addition of prill fat and prill fat along with rumen protected choline chloride resulted in improved body condition score and added income of Rs.7.14 and 18.76 per animal per day, respectively, in Murrah buffaloes.

The study revealed that supplementing prill fat in the ration of Murrah buffaloes helped in improving milk yield and fat per cent, which can be further enhanced by supplementing the ration with rumen protected choline chloride.

6.3 Suggestion for Further Work

- Large scale trial under field and farm conditions covering entire lactation needs to be carried out in lactating animals to confirm and validate the results of this study.
- Effect of long term feeding of prill fat and protected choline on reproductive performance of animals should be studied.

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