

*Research Article***Performance of Dairy Cows Fed Diet Supplemented with Cattle Biscuit**

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ABSTRACT

The study was carried out in Wahed Dairy Farm, Chattogram, Bangladesh for a period of 60 days to measure the performance of dairy cows fed diet supplemented with cattle biscuit (CB). Forty Local × Holstein Friesian crossbred milking cows were selected according to age, live weight, body condition score (BCS) and daily milk yield. Animals were randomly distributed into five dietary treatment groups designated as T₀ (Diet without CB), T₁ (Diet containing 0% urea supplemented CB), T₂ (Diet containing 25% urea supplemented CB), T₃ (Diet containing 35% urea supplemented CB) and T₄ (Diet containing 45% urea supplemented CB). Each treatment was divided into four replicates having two animals per replicate. All animals were stall fed. Results indicated that, daily milk yield of the cows in the treatment groups supplemented with CB significantly ($p < 0.05$) increased from 5th to 8th weeks. The highest average milk yield (8.3 kg/d) was recorded in T₂ group and the lowest milk yield (6.3 kg/d) was recorded in T₀ group. Fat percent of milk significantly ($p < 0.05$) increased during 1st, 2nd, 3rd, 7th and 8th weeks in the treatment groups compared to control group. Similar to fat, protein percent of milk also increased significantly in the 1st ($p < 0.001$); 2nd, 5th, 8th ($p < 0.05$) and

7th ($p < 0.01$) weeks. The SNF percent differed significantly in the 1st, 2nd, 5th ($p < 0.01$); 3rd and 7th ($p < 0.001$) weeks. The TS percent differed significantly in the 1st, 7th ($p < 0.01$); 3rd ($p < 0.001$) and 5th ($p < 0.05$) weeks in irregular fashion. On average (1-8 weeks), milk fat, milk protein, SNF and TS percent were higher in the T₂ and lower in T₀ groups respectively. Cholesterol, SGOT, SGPT, bilirubin, urea, creatinine and serum protein remained unchanged ($p > 0.05$) throughout the experimental period except serum glucose which differed ($p < 0.001$) in the 8th week. It was concluded that, supplementation of CB containing 25% urea substantially improved milk yield, milk fat, protein, SNF and TS without interfering hemato-biochemical parameters of the cows. Therefore, CB in addition to basal diet, may be suggested as a novel approach for feeding dairy cows.

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1. INTRODUCTION

In Bangladesh, a major constraint of dairy farming is the scarcity of feeds and fodders both in terms of quality and quantity (Quddus, 2012; Hamid, 2019; Rahman, 2019). Due to increased crop cultivation, farmers cannot spare lands for fodder production (FAO, 2016; Jalal et al., 2016; Dagar, 2017). As a result, dairy farming subsists mainly on straw based diet with limited supplementation of green fodder and little or no concentrate (Khan et al., 2009). Rice straw is an important crop residue contributing more than 90% of the total dry matter available to the dairy cattle (Jackson, 1977; Drake et al., 2002; Sarnklong et al., 2010). However, rice straw is deficient in protein and mineral content (Karim, 1988) and its cellulose and hemi-cellulose are poorly digested in the rumen (Jackson, 1977). Traditional urea molasses multi-nutrient block is recommended mainly for the animals that are fed with poor quality roughages like rice straw or mature grass because they generally contain less energy

and protein (Alam et al., 2006). Urea supplies readily available nitrogen to the microbes in the rumen and this nitrogen is used by them to produce protein for growth and production of the animal (Tiwari et al., 1990). Rumen microbes use molasses as a source of energy (Sansoucy et al., 1988; Luc et al., 2009). The supply of nitrogen, energy and sulfur from block increases the rate of degradation of fibrous substances present in rice straw and natural grass, which are utilized by the animal for better performance (Alam et al., 2006).

Supplementation of basal diet with urea molasses block is a common practice in Bangladesh which has shown to have beneficial effects in terms of feed intake, nutrient utilization, growth performance, milk yield and milk composition in dairy cows (Miah et al., 2000; Hosamani et al., 2003; Chowdhury et al., 2004; Alam et al., 2006; Ferdous *et al.*, 2007; Wanapat et al., 2013; Croft et al., 2014; Alam et al., 2016;

Lopez et al., 2016; Phesatcha and Wanapat, 2016).

However, preparation of block for animal is an obsolete and tedious process. It is not manufactured in large scale industrial volume for commercial dairy farms because of difficulty in preservation due to high moisture content and short shelf life. On the other hand, cattle biscuit, is a new concept which can be prepared commercially in industrial level and preserved for comparatively long period of time. Therefore, the present study was conducted to develop cost effective energy-protein rich CB and investigate the effects of supplementing CB on milk yield, milk composition and hemato-biochemical parameters of crossbred dairy cows.

2. MATERIALS AND METHODS

Study area

The study was carried out in Wahed Dairy Farm, Patiya, Chattogram, Bangladesh from September to October 2014. Ambient temperature ranged from 23.9° C to 31.6° C, humidity of 81-83% and rainfall 184.8-259.3 mm in the study area during the study period (BMD, 2014).

Experimental animals

Forty Local × Holstein Friesian (F₂) milking cows were selected purposively based on age, live weight, body condition score, daily milk yield, parity and stage of lactation. Individual pedigree of the experimental animals was collected from

the record sheet. All animals had a body condition score of 3-4 in a 5 scale.

Design of the experiment

Forty animals were randomly distributed in a completely randomized design into five dietary treatment groups designated as T₀ (Diet without CB), T₁ (Diet containing 0% urea supplemented CB), T₂ (Diet containing 25% urea supplemented CB), T₃ (Diet containing 35% urea supplemented CB) and T₄ (Diet containing 45% urea supplemented CB). Each treatment was divided into four replicates having two animals per replicate.

Management of animals

The animals were kept in single row face out system stanchion barn with well-ventilated condition and sufficient space (17.0×3.5 sft) per animal to keep them comfortable. All animals under the experiment were given an ear tag with identity number. Animals were cleaned regularly by a hose pipe with fresh water. A good sanitary condition was maintained throughout the experimental period. Milking was done twice a day (8.30 am and 4.30 pm) regularly. During milking, workers were maintained proper bio-security to guarantee good quality milk.

Preparation of CB

CB was prepared initially with different combinations of ingredients for different treatment groups taking into consideration its palatability and acceptance to the animal. Finally, the following proportion of ingredients were used (Table 1):

Table 1. Composition of cattle biscuit

| Ingredients (%) | Dietary treatments | | | |
|-----------------|--------------------|----------------|----------------|----------------|
| | T ₁ | T ₂ | T ₃ | T ₄ |
| Urea | 0 | 25.0 | 35.0 | 45.0 |
| Molasses | 12.5 | 10.0 | 10.0 | 7.5 |
| Sugar | 5.0 | 5.0 | 5.0 | 5.0 |
| Wheat flour | 25.0 | 17.5 | 20.0 | 17.5 |
| Rice polish | 10.0 | 5.0 | 5.0 | 5.0 |
| Maize powder | 15.0 | 10.0 | 5.0 | 5.0 |
| Soybean meal | 15.0 | 15.0 | 10.0 | 7.5 |
| Rice powder | 10.0 | 5.0 | 5.0 | 0.0 |
| Common salt | 2.5 | 2.5 | 2.5 | 2.5 |
| Vitamin-mineral | 5.0 | 5.0 | 5.0 | 5.0 |
| Total | 100 | 100 | 100 | 100 |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB

Granulated urea was ground into fine powder in a hammer mill, dissolved in water, mixed with molasses and stirred for a homogenous dough. The dough was stored in a jar for overnight. Remaining ingredients were mixed in a jar in the next morning. The dough was placed on a mixing machine following 2000 rotation per minute. The dough was placed into the

molding machine and forced into moulds where the negative shape of the dough pieces with patterns, name, type and docker holes were assigned. Excess dough scraped off with knife. CB was placed on a biscuit tray and dried. Artificial air flow was used to reduce the temperature. The CB was cooled, packed in an airtight packet for preservation.

**Figure 1.** Preparation of CB**Figure 2.** Heating tray for drying CB**Figure 3.** Packaging of CB**Figure 4.** Sampling of CB

Feeding of animals

All animals were stall fed in a single row face out system stanchion barn. Ration was supplied to the animal according to body maintenance and milk yield as per recommendation (ARC, 1980). All animals had free access to normal clean drinking water. CB was fed twice daily before milking in the morning and one hour before milking in the afternoon. Three CBs was given to the animals during every feeding time. Feed intake was recorded daily. The concentrate mixture consisted of rice polish (40.4 %), wheat bran (35.1%), broken maize (5.7%), soybean meal (5.7%), mustard oil cake (5.7%), mug powder (5.7%), dicalcium phosphate (0.28%), Vitamin premix (0.57%), common salt (0.85%).

Sampling of feed, blood and milk

Approximately 500 g of feed sample was taken daily and preserved in an air tightbag to analyze them in the laboratory as per AOAC (2006). Blood samples were collected directly from jugular vein through syringe and preserved in vacutainer tube. Samples were taken to the laboratory by using ice box and kept in a freezer at a temperature of -20°C. Approximately 4.0 ml blood was collected weekly from each animal up to 8 weeks. Milk samples were collected from individual animal. Total 40 samples were collected every week. Approximately, 200 ml of milk was collected by individual bottle, put in the icebox and immediately sent to the laboratory for analysis.

Analysis of milk sample

Milk samples were analyzed for fat, protein, lactose, total solids (TS), solids-not-fat (SNF) and mineral by using milk

analyzer (Lactostar, Funke-Gerber, Berlin, Germany). Lactostar adopted a combined thermo-optical procedure for determining milk components by measuring both thermal and optical qualities of the milk constituents. Before analysis, Lactostar was calibrated. After zero calibration, milk samples were analyzed and the results were recorded carefully in every week.

Analysis of blood sample

Clotted blood in the vacutainer tube was centrifuged at 3000 rpm for 20 minutes and prepared serum was collected into the eppendorf tube by micropipette. Sera were marked and stored in -20°C until analyzed for glucose, total protein, urea, creatinine, albumin, serum glutamic oxaloacetic transaminase (SGOT), serum glutamate-pyruvate transaminase (SGPT) by HumaLyzer 3000 (Wisbaden, Germany). Serum sample was mixed with the respective reagents in an ependroff tube. The serum with reagent was aspirated by spectrophotometric method to measure the serum parameter.

Statistical analysis

Data related to milk yield, milk composition and blood parameters were analyzed for ANOVA using Stata (Stata/SE 14.1, Stata Statistical Software, Stata Corporation, College Station, TX, USA). Means showing significant differences was compared by Dunnett Test. Statistical significance was accepted at $p < 0.05$.

3. RESULTS

Milk yield

Daily milk yield of the cows in the experimental groups (T₁, T₂, T₃ and T₄)

supplemented with different levels of CB had higher average milk yield (7.5, 8.3, 7.8 and 7.4 kg/d) than the control group (6.3 kg/d). Milk yield differed significantly ($p < 0.05$) from 5th to 8th week as the level of urea supplementation

increased in CB from 0 to 45%. From 1st to 8th weeks, highest average milk yield (8.3 kg/d) was recorded in T₂ group and the lowest average milk yield (6.3 kg/d) was recorded in the T₀ group (Table 2).

Table 2. Average milk yield (Kg/d/cow) of the cows fed diets supplemented with cattle biscuit

| Variable | Lactation | Dietary treatment | | | | | SEM | Sig. |
|------------|-------------------------------------|-------------------|----------------|----------------|----------------|----------------|-----|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Milk yield | 1 st wk | 6.3 | 7.3 | 7.4 | 7.0 | 6.5 | 0.2 | NS |
| | 2 nd wk | 6.4 | 7.3 | 7.7 | 7.3 | 6.7 | 0.2 | NS |
| | 3 rd wk | 6.4 | 7.3 | 8.1 | 7.5 | 7.0 | 0.2 | NS |
| | 4 th wk | 6.3 | 7.5 | 8.2 | 7.8 | 7.3 | 0.2 | NS |
| | 5 th wk | 6.3 | 7.7 | 8.3 | 8.0 | 7.5 | 0.2 | * |
| | 6 th wk | 6.1 | 7.6 | 8.7 | 8.0 | 7.9 | 0.3 | * |
| | 7 th wk | 6.5 | 7.7 | 8.9 | 8.2 | 8.1 | 0.3 | * |
| | 8 th wk | 6.4 | 7.9 | 8.9 | 8.3 | 8.2 | 0.3 | * |
| | 1 st -8 th wk | 6.3 | 7.5 | 8.3 | 7.8 | 7.4 | 0.2 | * |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant ($p > 0.05$); *=Significant ($p < 0.05$)

Milk fat

Milk fat percentage of the experimental cows varied in an irregular fashion during study period (Table 3). It was found that, fat percent in milk significantly ($p < 0.05$) increased during 1st, 2nd, 3rd, 7th and 8th weeks in the dietary treatment groups. However, the trend remained unchanged

($p > 0.05$) during 4th, 5th and 6th weeks. The highest milk fat (5.4%) was recorded in the 2nd week in T₂ group. The lowest milk fat (2.7%) was recorded in 1st week in T₁ and T₂ groups. On average, from 1st to 8th weeks, the best average milk fat (3.9%) was recorded in T₂ group.

Table 3. Milk fat (g/100 g) of the cows fed diets supplemented with cattle biscuit

| Variable | Lactation | Dietary treatment | | | | | SEM | Sig. |
|----------|-------------------------------------|-------------------|----------------|----------------|----------------|----------------|-----|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Milk fat | 1 st wk | 3.2 | 2.7 | 2.7 | 3.3 | 3.4 | 0.1 | * |
| | 2 nd wk | 4.7 | 5.3 | 5.4 | 4.6 | 4.4 | 0.2 | * |
| | 3 rd wk | 3.2 | 3.3 | 3.4 | 3.5 | 3.5 | 0.0 | * |
| | 4 th wk | 4.4 | 3.7 | 4.0 | 4.0 | 3.6 | 0.1 | NS |
| | 5 th wk | 3.3 | 3.3 | 3.4 | 3.5 | 3.5 | 0.0 | NS |
| | 6 th wk | 3.5 | 3.3 | 5.0 | 3.8 | 3.3 | 0.3 | NS |
| | 7 th wk | 3.3 | 3.3 | 3.4 | 3.6 | 3.7 | 0.1 | * |
| | 8 th wk | 3.0 | 3.3 | 3.8 | 3.4 | 3.3 | 0.1 | * |
| | 1 st -8 th wk | 3.6 | 3.5 | 3.9 | 3.7 | 3.6 | 0.1 | * |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant ($p > 0.05$); *=Significant ($p < 0.05$)

Milk protein

The protein percent of milk increased significantly in the 1st ($p<0.001$); 2nd, 5th, 8th ($p<0.05$) and 7th ($p<0.01$) week (Table 4). The highest protein percent (4.1%) was recorded in the 8th week in T₂ group and the lowest protein percent (2.2%) was

recorded in the 1st week in T₁ group. From 1st to 8th weeks, the highest average protein percentage (3.5%) was recorded in the T₁ and T₂ group and lowest (3.2%) in the T₀ group during 1st to 8th weeks of experimental period.

Table 4. Milk protein (g/100 g) of the cows fed diets supplemented with cattle biscuit

| Variable | Lactation | Dietary treatment | | | | | SEM | Sig. |
|--------------|-------------------------------------|-------------------|----------------|----------------|----------------|----------------|-----|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Milk protein | 1 st wk | 2.6 | 2.2 | 3.1 | 2.9 | 3.4 | 0.1 | *** |
| | 2 nd wk | 3.5 | 4.0 | 4.0 | 3.5 | 3.9 | 0.2 | * |
| | 3 rd wk | 3.0 | 3.2 | 3.2 | 3.3 | 3.3 | 0.0 | NS |
| | 4 th wk | 3.5 | 3.3 | 3.4 | 3.4 | 3.2 | 0.0 | NS |
| | 5 th wk | 3.1 | 3.2 | 3.2 | 3.4 | 3.2 | 0.0 | * |
| | 6 th wk | 3.6 | 3.6 | 3.8 | 3.6 | 3.5 | 0.0 | NS |
| | 7 th wk | 2.9 | 3.4 | 3.3 | 3.5 | 3.7 | 0.1 | ** |
| | 8 th wk | 3.3 | 3.2 | 4.1 | 3.2 | 3.3 | 0.1 | * |
| | 1 st -8 th wk | 3.2 | 3.5 | 3.5 | 3.4 | 3.4 | 0.1 | * |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant ($p>0.05$); *=Significant ($p<0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)

Milk SNF

The SNF percent differed significantly in the 1st, 2nd and 5th ($p<0.01$); 3rd and 7th ($p<0.001$) weeks (Table 5). However, it remained unchanged ($p>0.05$) for the 4th, 6th and 8th weeks. The highest SNF percent

(9.7%) was recorded in the T₁ group in 2nd week. The lowest SNF percent (8.3%) was recorded in the T₃ and T₄ groups in 8th and 4th weeks respectively.

Table 5. Milk SNF (g/100 g) of the cows fed diets supplemented with cattle biscuit

| Variable | Lactation | Dietary treatment | | | | | SEM | Sig. |
|----------|-------------------------------------|-------------------|----------------|----------------|----------------|----------------|-----|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Milk SNF | 1 st wk | 8.9 | 8.8 | 8.9 | 9.0 | 9.1 | 0.0 | ** |
| | 2 nd wk | 9.3 | 9.7 | 9.5 | 9.1 | 9.2 | 0.1 | ** |
| | 3 rd wk | 8.8 | 8.9 | 8.9 | 9.0 | 9.0 | 0.0 | *** |
| | 4 th wk | 8.9 | 8.6 | 8.7 | 8.7 | 8.3 | 0.1 | NS |
| | 5 th wk | 8.9 | 8.9 | 8.9 | 9.1 | 9.1 | 0.0 | ** |
| | 6 th wk | 9.0 | 9.1 | 9.5 | 9.0 | 8.9 | 0.1 | NS |
| | 7 th wk | 8.9 | 8.9 | 8.9 | 9.0 | 9.2 | 0.0 | *** |
| | 8 th wk | 8.6 | 8.4 | 8.8 | 8.3 | 8.7 | 0.1 | NS |
| | 1 st -8 th wk | 8.9 | 8.9 | 9.0 | 8.9 | 8.9 | 0.1 | NS |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant ($p>0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)

Milk TS

TS percent of milk differed significantly in the 1st, 7th ($p<0.01$); 3rd ($p<0.001$) and 5th ($p<0.05$) weeks (Table 6). The highest TS percent (15.5%) was estimated in the T₁ group in 2nd week. The lowest TS percent

(11.5%) was estimated in the T₁ group in the 1st week. From 1st to 8th week, the highest average TS (12.9%) were found in T₂ group.

Table 6. Milk TS (g/100 g) of the cows fed diets supplemented with cattle biscuit

| Variable | Lactation | Dietary treatment | | | | | SEM | Sig. |
|----------|-------------------------------------|-------------------|----------------|----------------|----------------|----------------|-----|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Milk TS | 1 st wk | 12.0 | 11.5 | 11.6 | 12.4 | 12.5 | 0.1 | ** |
| | 2 nd wk | 13.9 | 15.5 | 14.8 | 13.7 | 13.9 | 0.3 | NS |
| | 3 rd wk | 12.1 | 12.2 | 12.3 | 12.5 | 12.6 | 0.0 | *** |
| | 4 th wk | 13.3 | 12.3 | 12.7 | 12.7 | 11.9 | 0.1 | NS |
| | 5 th wk | 12.1 | 12.2 | 12.3 | 12.5 | 12.6 | 0.1 | * |
| | 6 th wk | 12.5 | 12.4 | 14.6 | 12.8 | 12.2 | 0.0 | NS |
| | 7 th wk | 12.2 | 12.1 | 12.4 | 12.6 | 12.9 | 0.1 | ** |
| | 8 th wk | 11.6 | 11.6 | 12.6 | 11.6 | 12.0 | 0.1 | NS |
| | 1 st -8 th wk | 12.5 | 12.5 | 12.9 | 12.6 | 12.6 | 0.1 | NS |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant ($p>0.05$); *=Significant ($p<0.05$); **=Significant ($p<0.01$); ***=Significant ($p<0.001$)

Blood parameters

There was no significant difference ($p>0.05$) in serum cholesterol. However, cholesterol level was moderately higher than the normal level both in the treatment and controls groups (Table 7). The highest average value of serum cholesterol (301.8) was found in T₂ group whereas the lowest value (285.2) was found in the T₃ group during 1st to 8th weeks. The SGOT appeared statistically non-significant ($p>0.05$) throughout the whole experimental period. However, the level of SGOT was typical in the treatment group. At the end of the experiment, highest serum SGOT (102.8 U/L) was found in T₂ group whereas the lowest value (75.3 U/L) found in T₃ group but both of them were in normal range. Similar to SGOT, SGPT remained unchanged ($p>0.05$) throughout the whole

experimental period. The highest level of SGPT (31.0 U/L) was found in T₃ group and the lowest level (21.4 U/L) was found in T₂ group. The level of SGPT was typical in the entire dietary treatment groups irrespective of CB supplementation.

Serum bilirubin appeared normal and did not differ significantly ($p>0.05$) from 1st to 8th weeks. The highest average serum bilirubin (0.20 mg/dl) was found in the T₄ group and lowest average (0.10 mg/dl) was found in the T₁ group. Serum urea level remained typical throughout the whole experimental period. The highest average value of serum urea (21.1 mg/dl) was found in the T₃ group and the lowest average value of serum urea (11.2 mg/dl) was found in the T₀ group.

Table 7. Cholesterol level (mg/dl), SGOT (U/L), SGPT (U/L), bilirubin (mg/dl), urea (mg/dl), creatinine (mg/dl), serum protein (g/dl) and serum glucose (mg/dl) of the experimental cows fed diets supplemented with cattle biscuit

| Parameter | Wks | Dietary treatments | | | | | SEM | Sig. |
|---------------|-----------------|--------------------|----------------|----------------|----------------|----------------|------|------|
| | | T ₀ | T ₁ | T ₂ | T ₃ | T ₄ | | |
| Cholesterol | 2 nd | 272.3 | 321.2 | 306.5 | 290.9 | 311.6 | 9.6 | NS |
| | 4 th | 316.3 | 297.3 | 286.6 | 300.2 | 309.8 | 5.7 | NS |
| | 6 th | 287.7 | 326.9 | 315.0 | 232.9 | 274.8 | 18.5 | NS |
| | 8 th | 252.4 | 330.6 | 252.1 | 296.4 | 278.9 | 16.5 | NS |
| SGOT | 2 nd | 142.9 | 152.9 | 101.9 | 77.6 | 74.5 | 18.2 | NS |
| | 4 th | 66.2 | 54.8 | 86.8 | 69.9 | 89.9 | 7.3 | NS |
| | 6 th | 111.5 | 76.3 | 73.4 | 108.3 | 65.4 | 10.7 | NS |
| | 8 th | 88.8 | 84.4 | 102.8 | 75.3 | 78.3 | 5.4 | NS |
| SGPT | 2 nd | 29.3 | 35.9 | 36.8 | 23.5 | 26.6 | 2.9 | NS |
| | 4 th | 37.8 | 27.1 | 42.0 | 40.5 | 37.7 | 2.9 | NS |
| | 6 th | 31.6 | 29.6 | 43.3 | 35.2 | 31.6 | 2.7 | NS |
| | 8 th | 28.7 | 26.9 | 21.7 | 31.0 | 30.4 | 1.9 | NS |
| Bilirubin | 2 nd | 0.12 | 0.15 | 0.15 | 0.15 | 0.15 | 0.00 | NS |
| | 4 th | 0.18 | 0.15 | 0.14 | 0.17 | 0.20 | 0.10 | NS |
| | 6 th | 0.14 | 0.20 | 0.20 | 0.15 | 0.20 | 3.10 | NS |
| | 8 th | 0.15 | 0.10 | 0.15 | 0.15 | 0.20 | 0.00 | NS |
| Urea | 2 nd | 32.6 | 35.7 | 29.6 | 29.3 | 12.7 | 3.1 | NS |
| | 4 th | 17.2 | 12.4 | 13.8 | 19.1 | 17.5 | 1.4 | NS |
| | 6 th | 12.4 | 20.6 | 17.2 | 10.8 | 30.5 | 3.1 | NS |
| | 8 th | 11.2 | 20.4 | 19.0 | 21.1 | 19.1 | 1.7 | NS |
| Creatinine | 2 nd | 3.4 | 3.4 | 2.2 | 2.4 | 2.9 | 0.2 | ** |
| | 4 th | 1.1 | 1.3 | 1.1 | 1.3 | 1.5 | 0.1 | NS |
| | 6 th | 1.3 | 1.7 | 2.0 | 1.6 | 1.9 | 0.1 | NS |
| | 8 th | 2.4 | 1.1 | 1.2 | 1.7 | 1.9 | 0.2 | NS |
| Serum protein | 2 nd | 8.9 | 11.5 | 10.6 | 11.5 | 12.1 | 0.5 | NS |
| | 4 th | 8.6 | 8.3 | 8.7 | 9.8 | 10.5 | 0.6 | NS |
| | 6 th | 8.9 | 9.4 | 9.1 | 9.4 | 12.8 | 0.8 | NS |
| | 8 th | 10.0 | 13.5 | 13.4 | 13.1 | 12.6 | 0.8 | NS |
| Serum glucose | 2 nd | 46.3 | 47.3 | 52.8 | 47.2 | 68.9 | 6.8 | NS |
| | 4 th | 77.3 | 85.6 | 84.6 | 62.0 | 75.0 | 8.3 | NS |
| | 6 th | 71.3 | 85.4 | 68.2 | 80.5 | 87.2 | 7.8 | NS |
| | 8 th | 75.0 | 71.8 | 68.9 | 76.0 | 85.0 | 10.4 | *** |

T₀=Diet without CB; T₁=Diet containing 0% urea supplemented CB; T₂=Diet containing 25% urea supplemented CB; T₃=Diet containing 35% urea supplemented CB; T₄=Diet containing 45% urea supplemented CB; SEM=Standard error of the mean; NS=Non-significant (p>0.05); **=Significant (p<0.01), ***=Significant (p<0.001)

Serum creatinine differed (p<0.01) only in the 2nd week. The highest average value (2.4 mg/dl) was recorded in T₀ group and the lowest average value (1.1 mg/dl) was found in the T₁ group. Serum protein did not differ significantly (p>0.05). The

maximum average value of serum protein (13.5 g/dl) was observed in T₁ group and the minimum average value (10.0 g/dl) was observed in the T₀ group. Unlike other serum parameters, glucose level strongly differed (p<0.001) in the 8th week.

The highest average value (85.0 mg/dl) was recorded in T₄ group and the lowest average value (71.8 mg/dl) found in the T₁ group.

A strong (-0.88), significant (p<0.01) and negative correlation was observed

between urea and glucose (Table 8). Significant (p<0.05) negative correlation (-0.74) was also observed between creatinine and glucose. On the other hand, cholesterol, SGPT, SGOT, bilirubin and serum protein were not significantly (p<0.05) correlated with each other.

Table 8. Multiple correlation co-efficient matrix of blood parameters of the cows fed diets supplemented with cattle biscuit

| Parameter | Cholesterol | SGOT | SGPT | Bilirubin | Urea | Creatinine | T. protein | Glucose |
|---------------|-------------|-------|-------|-----------|---------|------------|------------|---------|
| Cholesterol | 1.00 | | | | | | | |
| SGOT | 0.16 | 1.00 | | | | | | |
| SGPT | 0.67 | -0.36 | 1.00 | | | | | |
| Bilirubin | 0.20 | -0.21 | -0.09 | 1.00 | | | | |
| Urea | 0.08 | 0.51 | -0.07 | -0.30 | 1.00 | | | |
| Creatinine | 0.01 | 0.31 | -0.05 | 0.26 | 0.69 | 1.00 | | |
| Total protein | -0.07 | 0.34 | -0.57 | -0.21 | 0.14 | -0.37 | 1.00 | |
| Glucose | 0.10 | -0.42 | 0.05 | 0.41 | -0.88** | -0.74* | 0.06 | 1.00 |

SGOT=Serum glutamic oxaloacetic transaminase; SGPT=Serum glutamate-pyruvate transaminase

*=Significant (p<0.05); **=Significant (p<0.01)

4. DISCUSSION

In present study, the effect of CB on milk yield, milk composition and blood parameters were investigated. It was evident that, milk yield differed significantly (p<0.05) in the treatment groups for the last four weeks. Similar results were reported by Mapato et al. (2010) and Vu et al. (1999) who obtained better daily milk yield in crossbred Holstein-Friesian cattle. Increased milk yield in crossbred cows was also in close agreement with other investigators (Mazed, 1997; Miah et al., 2000; Chowdhury, 2004; Alam et al., 2006; Ferdous et al., 2007). The inherent mechanism for increased milk yield was described by Wanapat (1999) who reported that, UTRS improved digestibility of nutrients, feed intake and fermentation end products which in terms

resulted in increased milk production. However, in contrast with previous findings, Wanapat et al. (2009) did not find any change (p>0.05) in milk yield by using treated rice straw with urea or urea and calcium hydroxide. Similarly, using conventional urea at different levels in dietary ration, Erb et al. (1975) did not find any difference in milk yield.

In current study, milk yield was similar (p>0.05) for the first four weeks because the cows might have used additional dietary protein for improving previously deficient body condition score (Rafiq et al., 2007). However, significant (p<0.05) changes in milk yield at later stages were evident due to the effect of CB (Akter et al., 2004). The best average milk yield was obtained from cows fed 35% urea supplemented CB which could be due to the amount of blended cereal grains and molasses used in that group which

released urea slowly and provided rumen microbes sufficient energy and minerals to utilize urea offered through CB (Helmer et al., 1971). It could also be inferred that, appropriate combination and composition of the nutrients specially energy, protein, fat and minerals in the CB used in 25% urea supplemented CB group might have triggered cows to exhibit best milk yield (Colmenero and Broderick, 2006).

Fat percent in milk increased due to urea supplementation of CB. Lock and Shingfield (2003) stated that, starch was converted to acetyl Co-A through TCA cycle and joined fatty acid pool to form milk fat. Therefore, lactating cow fed CB had better performance in terms of fat percent in milk. This is the reason why the lowest fat percent was found in control group. In another study, Gonçalves et al. (2014) reported reasonably fair fat percent of milk (4.0%) with the experimental diet using 100% conventional urea. However, Casper and Schingoethe (1986) reported an unexplained decline in milk fat percentage for cows fed urea. In contrast, other investigators (Van Horn et al., 1967; Van Horn and Mudd, 1971; Galo et al., 2003) reported that, the milk fat percentage was unchanged by addition of urea in the diet of lactating cow. Santos et al. (2011) reported no difference in milk composition of cows fed diets with different levels of urea.

Susmel et al. (1995) and Wanapat et al. (2009) reported that, milk protein increased significantly in urea supplemented diets. Mba et al. (1975) showed that, urea-treated straw increased milk protein concentrations. Similar results were obtained by Wanapat *et al.* (2009). These observations are in close agreement with current finding. However,

Inostroza et al. (2010) found that, milk protein was unaffected ($p>0.10$) by treatment with urea containing feed. Milk protein significantly increased due to CB supplementation which provided sufficient N for the microbial protein synthesis. Another reason is that, the N supplied by CB was utilized by rumen microbes to synthesize available microbial protein which was broken down into amino acids in the gut. The portion of glucogenic amino acids along with other keto-acids worked as the precursor of serum glucose which was converted to blood glucose (McDonald et al., 2011). However, this observation is in disagreement with Debasis and Shingh (2003) who found no changes in the serum glucose when fed UMMB in lactating cow.

The SNF percent was unaffected ($p>0.05$) in the 4th, 6th and 8th weeks which is in well agreement with Wanapat et al. (2009). The TS percent was non-significant in 3rd, 4th, 6th and 8th weeks in the treatment groups compared to the control group. Similar finding was reported by Golombeski et al. (2006). The serum protein did not differ significantly ($p>0.05$) in the experimental period. Similar finding was observed by other investigators (Hosamani et al., 1998; Cenesiz et al., 2006). The total protein was higher than the normal value. Serum protein tended to increase due to the effect of CB. Hosamani et al. (2003) found higher serum total protein in the experimental group compared to control.

A key finding in renal disease is the elevation of serum creatinine. The majority of serum creatinine originates from the endogenous conversion of phosphocreatine in muscle. Creatinine is not reutilized in body. It is modified by

conditioning and muscle disease and distributed throughout the compartment of total body water. Creatinine concentration is not affected significantly by diet, protein catabolism and urinary flow. In present study, creatinine level significantly ($p < 0.01$) reduced in the 2nd and 3rd ($p < 0.05$) weeks indicating no renal disorders in the experimental cows.

The level of serum urea appeared constant ($p > 0.05$) during the study period and the value was normal till the end of the experiment which was in agreement with Radostits et al. (2006). Nozad et al. (2012) also reported similar results. Hosamani et al. (2003) found slightly higher urea level in treatment group compared to control. Serum bilirubin is derived from hemoglobin and is formed by macrophages and other leptomenigeal cells that degrade the hemoglobin from lysed red blood cells (Kaneko, 1997). In present study, no abnormal change in serum bilirubin was found indicating the proper hepatic function. During the entire experimental period, the total serum cholesterol was non-significant ($p > 0.05$). Similar finding was observed by other investigators (Cenesiz et al., 2006; Adedibu et al., 2013). The amount of total serum cholesterol was higher during the experimental period which could be due to the basal diet which contained cereal grains and succulent green grasses.

Liver is the main organ controlling metabolism in entire body. SGPT and SGOT are the specific enzymes of the liver which increases in the plasma by the destruction of the cell membrane and cell necrosis in acute liver disease and due to accumulation of toxic substances (Dunman and Erden, 2004). In present study, serum SGPT was normal and did

not differ significantly ($p > 0.05$) in the treatment groups feeding CB which is in well agreement with Cenesiz et al. (2006). Serum SGOT significantly ($p < 0.05$) decreased at the 5th week but remained within normal range among the treatment groups compared to control. It could be inferred that, the dietary urea supplementation by CB might have provided available amino acids for maintaining tissue repair which helped sustaining normal serum SGOT in the treatment groups. In close agreement with previous study, Cenesiz *et al.* (2006) did not find any change in SGOT in the treatment groups compared to control as in case of our study.

5. CONCLUSION

Milk production increased due to supplementation of CB without exhibiting harmful effects on blood parameters. The highest milk yield was recorded in the cows fed diet containing 25% urea supplemented CB. Similar to milk yield, fat, protein, SNF and TS content of milk substantially improved after feeding CB during study period in the experimental groups compared to control. Most of the serum parameters appeared normal in the treatment groups except cholesterol and protein. Wide range of CB supplementation did not influence normal level of serum bilirubin, SGPT and SGOT which indicated functional liver. Similarly, normal level of serum creatinine and urea reflected soundness of the functioning of kidney.

Recommendation

Most of the conventional methods for feeding urea used in Bangladesh or elsewhere are laborious and time

consuming. None of them are suitable either for long term preservation or for marketing. CB on the other hand, can be produced in large scale industrial level. It is convenient for transportation, feeding and storage as well. It can quickly be supplemented with basal diet as an additional source of protein, starch and mineral. Therefore, 25% urea supplemented CB may be suggested for the dairy farmer as a novel alternative to traditional urea supplements used for dairy cows.

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Author's Contribution

Md. Emran Hossain designed the study, supervised the research team and performed statistical analyses. Nahid Sultan conducted the animal trial, collected data and drafted the manuscript. Goutam Kumar Debnath performed all the milk tests. Omar Faruk Miazi, Shilpi Islam, Muhammad Yasin and Saidur Rahman provided insight in the research design. All authors read and approved the final manuscript.

Conflict of Interest

We declare that we have NO affiliations with or involvement in any financial, non-financial or professional organizations or

entity in the subject matter or materials discussed in this manuscript.

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