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# SUBSTITUTING CORN STEEP LIQUOR WITH UREA AS NITROGEN EQUIVALENCE ON NUTRIENTS INTAKE, DIGESTIBILITY, BLOOD BIOCHEMISTRY AND MILK COMPOSITION IN EARLY LACTATING NILI-RAVI BUFFALOES

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## ABSTRACT

This study was conducted to examine the influence of varying levels of corn steep liquor (CSL) on feed intake, nutrient digestibility, nitrogen balance, milk composition and blood biochemistry in early lactating *nili-ravi* buffaloes. Twenty five early lactating buffaloes were divided into five groups, 5 animals in each group, using Randomized Complete Block Design. The animals were divided into different blocks to account for the variation in their age, parity and physiological stage. Five *isonitogenous* (17% CP) and *isocaloric* (2.82 Mcal/kg) diets were formulated. The control diet (C) had 0% CSL and in CSL20, CSL40, CSL60 and CSL80 diets, 20, 40, 60 and 80% urea on nitrogen equivalent was replaced by CSL, respectively. Animals were fed twice daily at *ad libitum*. The daily feed offered and refusals were recorded to calculate dry matter intake (DMI). The sample of feed offered and refusal were used to determine dry matter (DM), crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). The DM, CP, NDF and ADF intake by buffaloes fed all diets remained unchanged (P>0.05). The DM and NDF digestibility were higher (P<.05) in animals fed diets containing CSL than those fed C diet. However, CP and ADF digestibility remained unaltered (P>0.05) across all diets. Plasma urea nitrogen (PUN) was lowest in buffaloes fed CSL80, CSL60 and CSL40 diets and was highest in buffaloes fed C diet. Nitrogen balance remained significant (P<0.05) higher in buffaloes fed CSL diets as compared to those fed C diet. However, there had been a non-significant difference in nitrogen balance in animals fed CSL20, CSL40 and CSL60 diets. Nitrogen intake, fecal nitrogen and urinary nitrogen values remained unchanged across all diets. The triiodothyronine ($T_3$), thyroxine ($T_4$) and their ratio remained unchanged (P>0.05) across all diets. Milk production, its fat, protein, true protein, non-protein nitrogen (NPN) and solid not fat values remained unaltered across all diets. The 4% fat corrected milk and lactose were higher (P<.05) in milk of buffaloes fed CSL40, CSL60 and CSL80 diets than those fed CSL20 and C diets. In conclusion, buffaloes fed diets containing CSL ate more DMI, had higher digestibility, better nitrogen balance, produced more milk and lower PUN than those fed C diet.

**Key words**: Corn Steep Liquor, Buffaloes, Blood Biochemistry, Milk production.

## INTRODUCTION

The dairy animals require maximum dry matter intake (DMI) to meet the increased nutrient requirement for enhanced milk yield. In developing countries, dairy animals derive their nutrient needs mainly from fibrous feed (Khan *et al*., 2004, Sarwar *et al*., 2002). Crop residues especially wheat and rice straws are being recognized most important contributors to dairy diets in developing regions of the world (Mehra *et al*., 2001, Man and Wiktorsson, 2001). However, their poor nutritive value and digestibility limit their intake and adversely affect animal performance.

In addition, increasing prices of feed ingredients has further worsened the feed availability situation. This ever widening gap can only be narrowed down by adding more nutrients in feedstuff inventory through nutritional evaluation. This will not only help abridge the nutrient shortage gap but will also make ration formulation more versatile. Ruminants are generally raised on natural pasture and crop residues which are of low protein, fermentable energy and other nutrients (Adugna and Sundstol, 2000). This results into low animal productivity because of reduced feed intake, digestibility, fermentation and microbial nitrogen. Unless we improve inherent poor nutritive characteristics of these feed resources (Nsahlai *et al*., 2000; Adugna and Sundstol, 2000), the animal productivity cannot be improved.

In developing countries, urea is generally fed to ruminants as an economical replacement for a part of protein in a ration. The amount of urea a ruminant animal can use depends on the digestible energy or total digestible nutrients (TDN) content of the ration (Whittier, 2014). Usage of urea as a source of $NH_3$ is not a perfect method, as the $NH_3$ liberated from urea because of action of ureolytic organism is not fully fixed in the straw.

One of the byproducts of corn industry is CSL which may offer a promising protein alternate (Nisa *et* *al*., 2004a) provided nutritionally evaluated. It is a byproduct of wet corn milling industry and is high (40%) in crude protein (CP). The CSL is a good source of carbohydrates, essential amino acids, peptides, organic compounds, magnesium, phosphorous, calcium, potassium, chloride, sodium, sulfur and myo-inositol phosphates (Nisa *et al*., 2004b, Hull *et al.*, 1996). It contains 50% dry matter (DM), 10% ash and 16% nitrogen free extract (NFE). Its pH is 3.7 and it contains 21% lactic acid (Khan *et al*., 2004). It is high in K which limited its inclusion in ruminant feed (Andrew and Tom, 2013) because K is bitter in taste that reduces feed consumption when high levels of CSL are used (Andrew and Tom, 2013). However, the scientific evidence regarding the influence feeding high level of dietary CSL on feed intake, digestibility and milk quality parameters in buffaloes is limited. Therefore, present study was planned to evaluate the effect of CSL on nutrients intake and their digestibility, nitrogen balance blood biochemistry, hormonal profile and milk composition in early lactating *nili-ravi* buffaloes.

## MATERIALS AND METHODS

**Animals and Diets:** Twenty five early lactating *nili-ravi* buffaloes were randomly divided into five groups, 5 animals in each group, using Randomized Complete Block Design. The animals were divided into different blocks to account for the variation in their age, parity, physiological stage days in milk. Five *isonitogenous* (17% CP) and *isocaloric* (2.82 Mcal/kg) diets were formulated. The control diet (C) had 0% CSL and in CSL20, CSL40, CSL60 and CSL80 diets, 20, 40, 60 and 80% urea on nitrogen equivalent was replaced by CSL, respectively (Table 1). Animals were treated against all internal and external parasites and vaccinated against local diseases (Hemorrhagic Septicemia, Food and Mouth Disease) before the start of experiment. The experiment lasted for 90 days.

**Feeding management and data collection:** Animals were housed on a concrete floor in separate pens and no mechanical means were used to control the house temperature. Relative humidity and temperature during the experiment remained 66.27±6.11% and 38.21±4.21°C, respectively. Animals were fed twice daily (0600 and 1400 h) at *ad libitum*. During collection period, samples of feed offered and refused were collected for analysis. Feed offered and refused were weighed to calculate the dry matter intake (DMI). The feces of each animal were collected daily in specially designed drum, weighed; mixed thoroughly and 20% of it was sampled and dried at 55°C. At the end of each collection period, dried fecal samples were composited by animal and 10% of the composited samples of each animal were taken for analysis. Before collection periods, the urine excreted by an animal was measured for three days to assess its volume in 24 hours. The reason for this practice was to know the amount of 50% $H_2SO_4$ to be added to maintain urine pH at about 4.0 which minimizes the escape of urinary ammonia nitrogen (Shahzad *et al*., 2008). This measured amount of 50% $H_2SO_4$ was added into cylinders and whole day urine excreted by a buffalo was recorded. After weighing the urine voided by each animal in 24 hour, 20% of it was sampled and preserved at -20°C (Shahzad *et al*., 2010). At the end of each collection period, the frozen urine samples were thawed and composited by animal and 10% of the composited urine sample was used for N analysis. Buffaloes were milked twice daily and individual milk weights were recorded. Milk was analyzed for CP, TP, non-protein nitrogen (NPN), glucose, fat, solid not fat (SNF) and total solids (TS) using Milko-Scan 33.

**Laboratory Analysis:** Dry matter was analyzed by drying it at 135°C until a constant weight was reached (AOAC, 1990, AOAC, 2002). Protein-N of the diets was analyzed using an acidified extract (20 g of fresh sample in 200 ml of 0.01 N HCl, agitated at 21°C for 22 h) and deproteinized with trichloracetic acid (TCA). Nitrogen fractions (Total-N, TCA insoluble-N) were done by the Kjeldahl method (AOAC, 1990, AOAC, 2002). Crude protein was calculated by multiplying %N with factor 6.25 (AOAC, 1990, AOAC, 2002). Acid detergent fiber (ADF) was determined using acetyl-trimethyl ammonium bromide detergent in 0.5 M sulfuric acid (Goering and Van Soest, 1970). Neutral detergent fiber (NDF) was determined using sodium sulfite (Van Soest *et al*., 1991). The analysis of milk constituent was performed on Milko-Scan 33 for the determination of fat, protein, true protein, NPN, lactose and SNF.

**Blood Sampling and Biochemical Analysis:** Blood samples were collected six hours after the last feeding on this trial. Blood sample (10 mL from each buffalo) was collected by puncturing jugular vein; 2mL was collected into the vaccutainers each containing 81µL of 15% EDTA (anticoagulant) solution, while 8mL was collected in test tube to harvest the serum for further analysis. Plasma samples were separated and frozen at -20°C within 60 minutes of collection.

**Statistical analysis:** The data were analyzed using a Randomized Complete Block Design. In cases of significance, means were separated by Duncan's Multiple Range Test (Steel *et al*., 1997) by using the SPSS (version 17).

## RESUTLS AND DISUCSSION

**Nutrient ingestion and digestibility:** The DMI, crude protein (CP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) intake remained unchanged

(P>0.05) across all diets (Table 2). The DM and NDF digestibilities were the highest (P<0.05) in animals fed CSL80 diet followed by CSL60, CSL40, CSL20 and C (Table 2) diets, respectively. However, the DM digestibility remained unchanged in animals fed CS80, CSL60 and CSL40 diets. Similarly, the DM digestibility remained unchanged for animals fed CSL20 and C diets. The CP and ADF digestibility by buffaloes fed diets containing CSL remained (P<0.05) unchanged (Table 2) across all diets.

**Nitrogen balance:** Plasma urea nitrogen (PUN) was lowest in buffaloes fed CSL diets and was highest (P<0.05) in buffaloes fed C diet (Table 3). Nitrogen balance remained higher (P<0.05) in buffaloes fed CSL diets than those fed C diet. However, there had been a non-significant difference in nitrogen balance in animals fed CSL20, CSL40 and CSL60 diets. Nitrogen intake, fecal nitrogen and urinary nitrogen values remained unchanged across all diets (Table 3).

**Thyroid hormones:** The triiodothyronine ($T_{3}$), thyroxine ($T_4$) and their ratio remained unaltered (p>0.05) in animals across all diets (Table 4). The higher values had been found in animals fed CSL diets as compared to those fed C diet.

**Milk yield and composition:** A non-significant (P>0.05) difference had been noticed in milk yield, milk fat, milk protein, milk true protein, milk non-protein nitrogen (NPN) and milk solid not fat (SNF) by animals fed CSL diets (Table 5). However, 4% fat corrected milk (FCM) and lactose values showed a significant difference in animals across all the diet. The highest (P<0.05) FCM value was observed in animals fed CSL60 and CSL80 diets followed by CSL40, CSL20 and C diets. The lactose value was higher (P<0.05) in buffaloes fed CSL80 and CSL60 diet followed by those fed CSL40, CSL20 and C diet, respectively.

**Nutrient ingestion and digestibility:** The DMI, CP, NDF and ADF intake by lactating buffaloes fed diets containing CSL and control diet remained unchanged and similar findings were reported by Yadave and Virk (1994), Sarwar *et al*. (1994), Dass *et al*. (2001) and Mehra *et al*. (2001). This lack of difference in nutrient intake may be because of similarity among dietary nutrients. However, Saadullah *et al*. (1981) reported higher DMI by cattle fed diets containing urea. The increased DM and NDF digestibility by buffaloes fed diets containing CSL may be because of its rapidly fermentable nature. The CSL might have ensured constant and sufficient nitrogen availability in the rumen compared to C diet containing only urea. This might have enhanced ruminal fermentation through increased microbial enzyme production leading to increased nutrient digestibility (Sarwar and Nisa, 1999; Sarwar *et al*., 2004). This implies that dietary cellulose and hemicellulose get extensively fermented, enhancing NDF and ADF digestibility in present study. The improved NDF digestibility can also be attributed to improved cellulytic activities in rumen. Nisa *et al*. (2004b) reported that the increased NDF digestibility could be because of its increased rate of degradation and shorter lag time of diets containing CSL than that of control diet.

**Nitrogen balance:** The highest nitrogen balance in buffaloes fed diet containing 80% CSL is an indication of better nitrogen utilization. The PUN was lower (P<0.05) in buffaloes fed diets with varying levels of CSL. Similarly, fecal N and urinary nitrogen were also non-significant in animals across all diets. Significantly less values of PUN in buffaloes fed varying levels of CSL has indicated maximum utilization of $NH_3$ for ruminal microbial growth (Sarwar *et al*., 2004). The slower release of fiber bound N was synchronized with fiber fermentation and thus utilized by the rumen micro-flora (Nisa *et al*., 2004b). Slow release of $NH_3$-N resulted in less $NH_3$ absorption through ruminal walls that consequently lowered the plasma urea N and urinary N loss and thus higher N retention in buffaloes. Sarwar *et al*. (1994) reported that rapid release of $NH_3$ in rumen was not efficiently utilized by ruminal microbes and absorbed into the blood through the ruminal walls thereby increasing plasma urea N and urinary N loss (Sarwar *et al*., 1994). Broderick *et al*. (1993) reported that blood urea was lower (3.8 vs. 5.2 mm) when true protein was fed than when the urea diet was fed. This finding probably was due to increased rate of ruminal $NH_3$ formation when urea was fed. The present results indicate that CSL is very effective in enhancing utilization of N by minimizing N loss at ruminal level. The low plasma N levels with CSL diets provide explanation of results of present study. It can be concluded that CSL diets slow down the release of ruminal $NH_3$ that maximizes N synchronization with carbon skeleton and this consequently minimizes N loss from the rumen (Sarwar *et al*., 2004).

**Thyroid Hormones:** The $T_3$, $T_4$ and their ratio remained unaltered by buffaloes across all diets. The mean values of serum $T_3$ and $T_4$ found in this experiment were within the normal physiological range (Table 4). The serum $T_3$ values were similar in all five groups. However, the values of $T_4$ were higher in animals fed CSL60 and CSL80 diets as compared to those fed other diets. This indicated a higher basal metabolic rate in animals fed CSL60 and CSL80 diets as compared to those fed other diets. An unaltered thyroid hormone level in animals across all diets is an indicator of no deleterious effect of CSL on different hormone levels in cow.

**Milk yield and Composition:** The highest milk yield (13.83 Kg/day) in buffalos fed CLS80 diet than those fed C (12.73 kg/day) diet supported the findings of Wanapat *et al*. (1985), Man and Wiktorsson (2001); Sutton (1987) and Cann *et al*. (1991). Sarwar *et al*. (2004) also reported that increasing dietary CSL might have provided a better nutrient synchrony at cellular level that helps synthesized more milk and milk constituents. The higher milk production by buffaloes may also be attributed to increased digestible NDF intake which provided sufficient energy to support the increased milk yield. Milk urea nitrogen (MUN) is milk quality indicator which equilibrates with and is proportion to blood urea nitrogen. So, this also indicates urea nitrogen status in dairy cows (Ahlam *et al*., 2010). The increased MUN generally results when blood urea nitrogen is high which adversely affects animal's reproductibility. The findings of present study were concordant to those reported by Sutton (1987) who noticed no change in milk protein concentration in buffaloes fed diets containing CSL. However, an increased milk protein tendency was noticed in buffalos fed diets containing high amount of CSL. True protein and NPN, as percentage of milk protein did not show any treatment effect. Percent milk fat, SNF and total solid remained unchanged across all treatments whereas lactose percentage showed a significant difference. The results of present study were consistent to those reported by Man and Wiktorsson (2001).

**Table 1. Ingredients and chemical composition of experimental diets**

| Ingredients (%) | Experimental Diets[1] | | | | |
|---|---|---|---|---|---|
| | C | CSL20 | CSL40 | CSL60 | CSL80 |
| Wheat Straw | 30.0 | 30.0 | 30.0 | 30.0 | 30.0 |
| Corn Grains | 25.0 | 24.0 | 22.0 | 22.0 | 20.0 |
| Urea | 4.0 | 3.0 | 2.0 | 1.0 | 0.0 |
| CSL | 0.0 | 5.0 | 10.0 | 15.0 | 20.0 |
| Canola Meal | 0.0 | 0.0 | 3.0 | 4.5 | 5.0 |
| Sunflower Meal | 0.0 | 0.0 | 3.0 | 4.5 | 4.0 |
| Corn Glutten 30% | 0.0 | 0.0 | 2.0 | 4.5 | 5.0 |
| Rice Polishings | 17.0 | 15.0 | 10.0 | 4.0 | 4.0 |
| Maize Bran | 15.0 | 14.0 | 9.0 | 4.0 | 3.0 |
| Enzose | 5.0 | 5.0 | 5.0 | 6.5 | 5.0 |
| $NaHCO_3$ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Salt | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| DCP | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| ***Analyzed Chemical Composition (%)*** | | | | | |
| Dry Matter | 90 | 90 | 89.9 | 89.8 | 88.8 |
| Crude Protein | 17 | 17 | 17 | 17 | 17 |
| Neutral Detergent Fiber | 32 | 32.1 | 32.1 | 32.2 | 32.3 |
| Acid Detergent Fiber | 24.5 | 24.6 | 24.7 | 24.8 | 25.6 |
| Non Structural Carbohydrates | 30 | 30 | 30 | 30 | 30 |
| Metabolizable Energy ME (Mcal/kg) | 2.82 | 2.82 | 2.82 | 2.82 | 2.82 |

[1]C, CSL20, CSL40, CSL60 and CSL80 diets contained corn steep liquor as replacement of urea at the rate of 0, 20, 40, 60 and 80% on the basis of nitrogen supply by corn steep liquor, respectively.

**Table 2. Effect of varying levels of corn steep liquor when replaced with urea on nutrient intake and their digestibility in buffaloes.**

| Parameters | Diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | C | CSL20 | CSL40 | CSL60 | CSL80 | SE |
| ***Nutrient intake (kg/day)*** | | | | | | |
| Dry matter | 17.61 | 17.50 | 17.52 | 17.45 | 17.50 | 0.026 |
| Crude protein | 3.00 | 2.98 | 2.98 | 2.97 | 2.98 | 0.022 |
| Neutral detergent fiber | 8.51 | 8.57 | 8.56 | 8.50 | 8.62 | 0.025 |
| Acid detergent fiber | 5.82 | 5.79 | 5.83 | 5.85 | 5.87 | 0.023 |
| ***Nutrient digestibilities*** | | | | | | |
| Dry matter | 64.6[bv] | 66.3[ab] | 67.1[ab] | 67.9[a] | 68.5[a] | 0.423 |
| Crude protein | 79.9 | 78.2 | 78.2 | 78.3 | 78.0 | 0.287 |
| Neutral detergent fiber | 58[c] | 59[ab] | 60[bc] | 61[b] | 62.3[a] | 0.457 |
| Acid detergent fiber | 43 | 43 | 44 | 44 | 44.5 | 1.021 |

Means within row bearing different superscripts differ significantly ($p<0.05$)

[1]C, CSL20, CSL40, CSL60 and CSL80 diets contained corn steep liquor as replacement of urea at the rate of 0, 20, 40, 60 and 80% on the basis of nitrogen supply by corn steep liquor, respectively.

**Table 3. Effect of varying levels of corn steep liquor when replaced with urea on nitrogen balance in buffaloes.**

| Parameters | Diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | C | CSL20 | CSL40 | CSL60 | CSL80 | SE |
| Plasma Urea Nitrogen, mg/dL | 17.8[a] | 16.8[b] | 16.2[bc] | 15.9[c] | 15.1[c] | 0.628 |
| Nitrogen Intake, g/day | 480 | 476.8 | 476.8 | 475.2 | 476.8 | 0.941 |
| Fecal Nitrogen, g/day | 416 | 412.8 | 412.8 | 411.2 | 411.2 | 0.233 |
| Urinary Nitrogen, g/day | 48.0 | 47.68 | 47.68 | 47.52 | 47.68 | 2.925 |
| Nitrogen Balance, g/day | 16[b] | 16.32[b] | 16.32[b] | 16.48[b] | 17.92[a] | 2.845 |

Means within row bearing different superscripts differ significantly ($p<0.05$)
[1]C, CSL20, CSL40, CSL60 and CSL80 diets contained corn steep liquor as replacement of urea at the rate of 0, 20, 40, 60 and 80% on the basis of nitrogen supply by corn steep liquor, respectively.

**Table 4. Effect of varying levels of corn steep liquor when replaced with urea on thyroid hormone profile in early lactating buffaloes.**

| Parameter | Diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | C | CSL20 | CSL40 | CSL60 | CSL80 | SE |
| **$T_3$** (nmol/L) | 1.75 | 1.8 | 1.85 | 1.9 | 1.8 | 0.025 |
| **$T_4$** (nmol/L) | 40.41 | 41.13 | 41.7 | 42.32 | 43.17 | 0.335 |
| **$T_3/T_4$** | 23.1 | 22.85 | 22.54 | 22.27 | 23.98 | 0.269 |

Means within row bearing different superscripts differ significantly ($p<0.05$)
[1]C, CSL20, CSL40, CSL60 and CSL80 diets contained corn steep liquor as replacement of urea at the rate of 0, 20, 40, 60 and 80% on the basis of nitrogen supply by corn steep liquor, respectively.
T3- triiodothyronine, T4- thyroxine

**Table 5. Effect of varying levels of corn steep liquor when replaced with urea on weight gain, milk quantity and milk composition in early lactating buffaloes.**

| Parameter | Diets[1] | | | | | |
|---|---|---|---|---|---|---|
| | C | CSL20 | CSL40 | CSL60 | CSL80 | SE |
| **Volume (kg/d)** | 7.6 | 7.6 | 7.7 | 7.7 | 7.8 | 0.277 |
| **4% FCM** | 12.73[c] | 12.92[bc] | 13.09[b] | 13.48[a] | 13.84[a] | 0.521 |
| **Fat (%)** | 6.7 | 6.8 | 6.8 | 7.0 | 7.1 | 0.236 |
| **Protein (%)** | 3.6 | 3.6 | 3.7 | 3.75 | 3.9 | 0.220 |
| **True Protein (%)** | 3.38 | 3.38 | 3.40 | 3.41 | 3.44 | 0.030 |
| **Non-Protein Nitrogen (%)** | 0.22 | 0.23 | 0.24 | 0.24 | 0.24 | 0.004 |
| **Lactose (%)** | 5.6[b] | 5.65[b] | 5.70[ab] | 5.80[a] | 5.95[a] | 0.039 |
| **SNF (%)** | 10.5 | 10.7 | 10.8 | 11.0 | 11.5 | 0.236 |

Means within row bearing different superscripts differ significantly ($p<0.05$)
[1]C, CSL20, CSL40, CSL60 and CSL80 diets contained corn steep liquor as replacement of urea at the rate of 0, 20, 40, 60 and 80% on the basis of nitrogen supply by corn steep liquor, respectively.
FCM-Fat Corrected Milk, SNF- Solid Not Fat

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