



Biochemical and Microbiological Characteristics of Indigenous and Crossbred Fresh Cow Urine

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ABSTRACT

The present study was conducted to examine biochemical and microbiological properties of fresh cow urine. A total of 98 fresh urine samples from apparently healthy indigenous and crossbred cows maintained under organized farming system were collected aseptically in sterile vials. The urine samples were subjected to biochemical, microbial, yeast and mould examination using respective diagnostic kits and suitable culture media. Average fresh urine pH was significantly ($p < 0.05$) higher in indigenous cows than in crossbred cows. No difference was observed for urea concentration between indigenous and crossbred cows. The average urea concentration was 1.56% in all the fresh urine samples collected from indigenous cows. Dry crossbred cows had significantly ($p < 0.01$) higher urea concentration than in the milch cows, however, no difference was observed in milch and dry indigenous cows. Milch indigenous cows had significantly ($p < 0.05$) lower creatinine concentration than in the dry cows. On microbial examination of different samples, bacterial growth was absent except four samples which showed bacterial colonies on BHI and MLA. The SDA method for fungal growth suggested no such growth in the study. The present study revealed that the fresh cow urine obtained from apparently healthy cows can be utilized for recommended preparations in agricultural operations.

HIGHLIGHTS

- The biochemical and microbiological characteristics of fresh urine collected from certain indigenous and crossbred cows were analyzed.
- Fresh cow urine free from microorganisms obtained from healthy cows can be utilized for recommended agricultural operations.

Keywords: Biochemical, Cattle, Indigenous, Microbiology, Urine

India is highest milk producing country in the world. According to 20th livestock census, the country possesses 193.47 million cattle of which 51.36 million are crossbred while the rest 142.11 million are Indigenous (DAHD, 2022-23). Cattle have about 52% contribution in total milk production of our country. Huge population of low and unproductive indigenous cattle has become a liability in the absence of proper utility of these animals. The demand of high yielding crossbreds and mechanization has kept the necessity of these animals a side. With the advancement in agriculture, the use of mechanical power in agriculture has increased but draught animal power

continues to be used on farms due to small land holdings and hill agriculture (Choudhary *et al.*, 2017). The efforts are being made through the use of waste/excreta from these animals preparing medicinal/agricultural/utility etc. products of general and specific in nature.

Cow urine has got several applications in agriculture

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and human ayurvedic medicines. An ancient literature in Ayurveda states that cow urine is one of the best natural remedies to cure many bacterial and fungal diseases (Edwin *et al.*, 2008). The cow urine contents are water 95%, urea 2.5%, minerals (iron, calcium, phosphorus, potassium), salt, hormones and enzymes 2.5% (Bhadauria, 2002). The cow urine has several biological activities such as antimicrobial, antidiabetic, antioxidant, antitumor, molluscicidal and others (Rakesh *et al.*, 2013).

Nitrogen content in urine plays a critical role in assessing the environmental impact of dairy farming (Danese *et al.*, 2024). The use of cow urine can be cost-effective and eco-friendly approach for controlling various diseases of plants (Gawade *et al.*, 2020). The urea present in cow urine can also be used for treatment of crop residues replacing commercially available urea (Saadullah *et al.*, 1980). However, several factors have been reported responsible for variation in the biochemical composition of cow urine collected from different types of cattle, moreover, scanty information is available on characteristics of fresh cow urine collected from crossbred cattle. Therefore, the present study examined biochemical and microbiological characteristics of indigenous and crossbred fresh cow urine.

MATERIALS AND METHODS

The study was conducted at cattle farm, Sardar Vallabh-Bhai Patel University of Agriculture and Technology, Modipuram, Animal Farm, ICAR-CIRC, Meerut and Indigenous cattle dairy farm, Panchli village of Meerut district. Fresh urine samples were collected from animals of different indigenous cattle breeds viz. Sahiwal, Tharparkar, Gir, Kankrej and Rathi, and Crossbred (Holstein Friesian × Sahiwal).

A total of ninety-eight urine samples from apparently healthy indigenous and crossbred cows maintained under these farms were collected aseptically in 50 ml sterile vials. The collected samples from animals were then transported to the laboratory under cold chain for further processing. The biochemical parameters (urea, creatinine and uric acid) were analysed using respective diagnostic kits.

For microbiological analysis, the urine samples were inoculated on Brain Heart Infusion agar and MacConkey Lactose Agar (MLA) for bacterial isolation and on

Sabouraud's Dextrose Agar (SDA) for detection and identification of fungal growth. The plates were incubated under aerobic conditions for 24-48 hours at 37°C for bacterial and yeast growth and at 27°C for the mould growth. After incubation each different colony was examined macroscopically (colony morphology and pigment production) and microscopically (Gram staining). Identification of microorganisms was done using conventional bacteriological methods according to standard procedures (Quinn *et al.* 2011).

The results obtained were subjected to statistical analysis (Snedecor and Cochran, 1994) followed by Duncan's multiple comparisons test (SPSS version 20). The significance was declared at $P < 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

The urine samples collected from all the animals belonging to both the breeds were of pale in colour. The cattle urine was normal in colour due to presence of urobilin (Reece, 2005). Similar observations were reported by the Kanaujia and Upadhyay (2018) where the fresh cow urine was pale yellow with light, strong and very strong odour.

Least square means with respect to pH, urea, creatinine and uric acid in both categories of animals are given in Table 1. Fresh cow urine pH varied from 7.31 to 8.42 in the urine samples of indigenous and crossbred cows. Average fresh urine pH was significantly ($p < 0.05$) higher in indigenous cows than in crossbred cows. Chitteni *et al.* (2022) reported 8.1-8.2 pH in Punganur cows. No significant change in urinary pH was observed between lactating and dry cows. Normal urine of cattle has an alkaline pH (Mavangira *et al.*, 2010). The results are in agreement with earlier report (Davis, 2004) who reported no significant difference between different age groups of Sahiwal and crossbreds. The average pH values obtained in this study are in agreement with Reece (2005) who reported the normal physiological range of pH as 7.0 to 8.4 for cattle. Similar results were obtained by Herman *et al.* (2019) in dairy cows and Ihedioha *et al.* (2019) in Nigeriantrade cattle.

No significant variations were observed for average urea, creatinine and uric acid values between indigenous and crossbred animals (Table 1). The average urea concentration was 1563.13 mg/dl in all the fresh urine

samples collected from indigenous cows. The average urea concentration in indigenous milch and dry cows was 1.59 and 1.33%, respectively. Dry Holstein × Sahiwal cows had significantly higher ($p < 0.05$) urine urea concentration than milch cows (Table 2).

Table 1: Least square means of fresh indigenous and crossbred cow urine parameters

Parameter	Indigenous cow	Crossbred cow
pH	7.81 ± 0.08 ^a	7.75 ± 0.08 ^b
Urea (mg/dl)	1563.13 ± 138.66	1367.96 ± 236.98
Creatinine (mg/dl)	102.98 ± 22.28	161.02 ± 29.23
Uric acid (mg/dl)	7.54 ± 1.22	8.24 ± 1.74

Figures with different superscripts differ significantly ($p < 0.05$) in rows.

Significantly high concentration of urea, uric acid, protein and creatinine were observed in urine of dry cows compared to heifers and lactating Punganur cows (Chitteni *et al.*, 2022). Similar values of urea and uric acid were reported by Ramani *et al.* (2012) in pregnant, milking cows and calves of Gir breed. Over feeding during dry period leads to nutrition and metabolic changes which may be the reason for high concentrations of urea in dry cows compared to lactating cow and heifers. Filipejova and Kavacik (2009) reported high concentration of urea during dry period compared to lactation in dairy cows.

The average creatinine level in Indigenous cattle was 102.98 mg/dl. Dry indigenous cows had significantly higher level of creatinine than milch cows. Creatinine concentration depends on the body hydration status, and, therefore, it has a diurnal excretion reflecting cow-to-cow

variability (Lee *et al.*, 2019). Presence of urea, creatinine and other chemical agents has strongly explained the antimicrobial and germicidal properties of cow urine (Achliya *et al.*, 2004).

Table 2: Least square means of milch and dry indigenous and crossbred cow urine parameters

Parameter	Indigenous cow		Crossbred cow	
	Milch	Dry	Milch	Dry
Urea (mg/dl)	1592.50 ± 132.45	1327.30 ± 95.57	1328.40 ± 193.65 ^b	1418.20 ± 63.42 ^a
Creatinine (mg/dl)	53.64 ± 7.69 ^b	144.93 ± 38.68 ^a	157.28 ± 17.19	172.02 ± 25.58

Figures with different superscripts differ significantly ($p < 0.05$) in rows.

In microbial examination of different urine samples, bacterial growth was absent in the samples collected, except few samples which showed bacterial colonies on BHI and MLA (Fig. 1 and 2). The colonies were confirmed under oil immersion microscope which suggested coccobacilli organisms. *Staphylococcus capitis*, *Staph. haemolyticus*, *Enterococcus faecalis* and *E. coli* were observed from the urine samples of four cows. The repeated samples from these cows showed presence of the same bacteria. No bacteria were found after autoclaving of these samples.

Ananda (2011) and Rawat *et al.* (2019) reported presence of bacterial population (260×10^4 cfu/ml and 2.12×10^3 cfu/ml, respectively) in cow urine. The presence of bacterial population in the urine might be due to non-sterile collection of urine samples and infected animal. The SDA method for fungal growth by loop streak method suggested



Fig. 1: Bacterial colonies on BHI and MLA

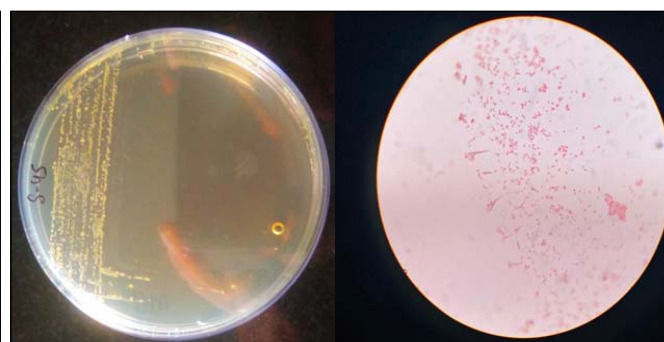


Fig. 2: Bacterial colonies (left); Coccobacilli (right)

no such growth in the present study. However, Rawat *et al.* (2019) reported 5.12 ± 0.49 (10^3 cfu/ml) count of yeast and mould in cow urine.

CONCLUSION

It may be concluded that variation in biochemical constituents in urine samples of some indigenous and crossbred cows may be due to different physiological and nutritional stages. The present study revealed that the fresh cow urine obtained from apparently healthy animals can be utilized for recommended preparations in agricultural operations.

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