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Effect of native and crossbreed cow urine distillate on haemato-biochemical profile of broilers

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Abstract
The present study was designed to investigate the changes in haemato-biochemical profile of broiler chickens during administration of cow urine distillate (CUD). The cow urine distillate was prepared in the laboratory and was evaluated for its physical and chemical composition. Eighty, day old broiler chicks were randomly assigned into four groups with twenty birds each and were acclimatized for four days before the start of cow urine distillate supplementation. Control group T-1 was given drinking water without cow urine distillate, while groups T-2, T-3 and T-4 were provided drinking water mixed with cow urine distillate of Ongole, Sahiwal and Holstein Friesian (H.F) crossbred, respectively @10ml/liter. Six birds from each group were randomly selected and slaughtered on 28 th day and 42nd day of experiment. Blood samples were collected from the sacrificed birds and were analyzed for haematological and biochemical parameters. At the end of the experimental trial i.e. at 42nd day, the mean values of red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were significantly increased in cow urine distillate treated groups compared to control, while the serum biochemical parameters showed no significant difference between groups.

Keywords: cow urine distillate, native breed, crossbreed, broilers, haemato-biochemical profile

Introduction
Composition and characteristics of cow urine were directly related to diet of animals (Burger and Smith, 1987) [2]. Haematological blood picture can give significant hints for the avian practitioner about anemia, dehydration, infection and Aspergillosis (Campbell, 1995) [3], and it helps in the early detection of diseases. Biochemical parameters play an assistant role for the faster and realistic evaluation of multiple organ dysfunctions (Huang *et al.*, 2018) [5].
'The cow' is a mobile medical dispensary and cow urine is a panacea of all diseases (Pathak and Kumar, 2003) [16]. Cow urine has been elaborately explained in Ayurveda and described in "*Sushrita Samhita" and "Ashtanga Sangraha"* to be the most effective substance of animal origin with innumerable therapeutic properties (Kekuda *et al.*, 2010) [9]. Cow urine distillate is a product of cow urine; it is prepared by condensing the vapors of fresh cow urine using glass distillation apparatus (Kadagi *et al.*, 2012) [7]. Most of the medicines are made by distilling urine and collecting ark which has longer shelf life than cow urine and it is the supreme vedic boon for maintaining holistic health. A very little information is available on the effect of cow urine distillate on haemato-biochemical profile in chicken, therefore, present study was undertaken to evaluate the influence of cow urine distillate on certain haematological and serum biochemical parameters in chicken.

Materials and Methods
Chemicals
Biochemical kits required for estimation were purchased from Biosystem diagnostics Pvt. Ltd.

Collection of urine and preparation of cow urine distillate (CUD)
Early morning first voided urine was collected and was filtered through muslin cloth, then the pooled sample from three day collection were subjected to distillation at 70°C using glass distillation apparatus. The obtained CUD was stored at -4^0 C for further use.

Physico-chemical composition of CUD
The physical parameters such as color, odour and transparency of CUD were recorded by sensory evaluation. pH and specific gravity were determined using URIT-50 Vet analyzer.

Chemical parameters such as calcium (O- Cresolphthalein method), phosphorus (Phosphomolybdate /UV method), protein (Pyrogallol Red method), urea (Urease method) and uric acid (Uricase/ Peroxidase method) were estimated using respective standard biosystem kits by A15 automated biochemical analyzer as per the manufacture's instruction.

Animals and Experimental conditions

One-day old broiler chicks (n=80) belonging to single hatch were procured from Balaji hatcheries, Chittoor and were randomly allotted to four treatment groups with twenty (20) birds in each group. The chicks were given four days to acclimatize to their new growing environment and 10ml of ketone body free CUD was administered per liter of drinking water starting from the fifth day to throughout the entire experimental period. Group T-1 was given normal drinking water and it served as control. Group T-2 and T-3 birds received drinking water mixed with CUD of indigenous breeds, Ongole and Sahiwal, respectively, whereas Group T-4 received drinking water mixed with CUD of H.F crossbreed cow.

The chicks were reared under deep litter system and were maintained under standard management conditions throughout the experimental period of 42 days. The experimental diet was provided according to the standards prescribed by Bureau of Indian Standards (B.I.S). The broiler starter and broiler finisher diets were fed *ad libitum* to the birds from 1 to 28 and 29 to 42 days of age, respectively.

Haematological and serum biochemical studies

Six birds from each group were randomly selected and sacrificed on 28th and 42nd day of experiment by decapitation using a sterile sharp knife and blood samples were collected in EDTA and clot activator vials. Blood collected in EDTA vials was used for haematological studies, whereas blood collected in clot activators were allowed to clot at room temperature and then subjected to centrifugation at 3000rpm for 10min for separation of serum.

Haematological parameters (total leucocyte count, lymphocyte count, monocyte count, granulocyte count, total erythrocyte count, hemoglobin, MCV, MCH, MCHC) were determined using Mindray Vet 2800 Haematology analyzer.

Serum biochemical constituents such as total protein by Biuret method, albumin by Bromocresol green method, triglycerides by Glycerol phosphate oxidase/Peroxidase method, total cholesterol by cholesterol oxidase/Peroxidase method, glucose by Glucose oxidase method, urea by urease method, creatinine by Jaffe's compensated method and ALT by IFCC method were estimated using A15 automated biochemical analyzer.

Statistical analysis

All the data have been presented as mean ±SE. Statistical comparisons were made using one way analysis of variance (ANOVA) by using computer software SPSS (Version 20). Significant differences ($p<0.05$) between different experimental groups were analyzed by Duncan's test.

Results and Discussion

Physicochemical composition of CUD

The physical and chemical composition of CUD was presented in Table 1.

Composition of urine varies according to the species, breed, season, physiological status, quality and quantity of feed and water consumed (Siener and Hesse, 2002) [19].

Physical composition

Food, metabolic products and medications can cause change in urine color (Simerville *et al.*, 2005) [20]. In the present study, the CUD prepared from both native (Ongole, Sahiwal) and crossbred (Holstein-Friesian) cows urine was watery with clear transparency and light odour, which was in agreement with the results of Kanaujia and Upadhyay (2018) [8] who reported that distillates prepared in lab were transparent and light odoured. On the contrary, yellow color of the CUD was observed by Thombre and Sapkal (2019) [24].

Urine pH is highly influenced by diet, recent feeding, bacterial infection, storage time, metabolic and respiratory alkalosis, urinary retention (Mavangira *et al.*, 2010) [13]. Specific gravity of urine depends on the concentration of solutes viz., chlorides, sulfates, phosphates, bicarbonates, urea and creatinine (Price *et al.*, 1940) [17]. The value of pH (7.5-8.0) and specific gravity (1.015-1.02) obtained in this study were in agreement with the normal physiological range of pH i.e. 7.0 to 8.4 and specific gravity i.e. 1.015 to 1.040 as reported by Reece (2005) [18] for cattle. Similarly, the findings obtained in the present study were comparable with the results of Manjramkar (2018) [10], who observed a specific gravity of 1.026±0.005 and mean pH of 8.36±0.09 for laboratory produced CUD.

Table 1: Physicochemical composition of CUD

Physical composition					
Breed	**Color**	**Odour**	**Transparency**	**pH**	**Specific gravity**
Ongole	Like water	Light odour	Transparent	7.5	1.015
Sahiwal	Like water	Light odour	Transparent	8	1.02
H.F	Like water	Light odour	Transparent	7.5	1.02
Chemical composition					
Breed	Calcium (mg/dl)	Phosphorus (mg/dl)	Protein (mg/l)	Urea (mg/dl)	Uric acid (mg/dl)
Ongole	5.61	7.54	88.83	345.92	22.91
Sahiwal	4.72	6.98	83.20	325.36	22.47
H.F	4.95	5.78	89.50	124.70	22.63

Chemical composition

Higher calcium, phosphorus and urea levels were noticed in Ongole CUD. Saline infusion or high dietary sodium content, high dietary protein, hypercalcemia, vitamin D deficiency or excess acidosis, mineralocorticoid/ glucocorticoid excess, osmotic diuretics and loop diuretics may be some of the factors that increase urinary calcium excretion; whereas parathyroid hormone, calcitonin, dehydration, alkalosis and thiazide diuretics can decrease the urinary calcium excretion (Melvin, 1992) [14]. The differences between high- and low-

phosphorus excreting cows may be due to differences in phosphorus reabsorption capacity (Manston and Vagg, 1970) [11]. Urea levels in urine depend on the dietary proteins but, urea can also be present in urine in complete absence of protein diet. This attributes to breaking down of tissue protein (Jindal and Singh, 2011) [6].

Higher total protein values were recorded for H.F cross bred CUD followed by Ongole and Sahiwal CUD. The observed values of proteins in the present study were within the normal physiological range and not indicative of proteinuria in accordance to Stojic (1982) [21] who reported normal urinary protein values in bovines as 64- 170mg/L.

Uric acid levels were almost similar in the all the three breeds CUD. Urinary excretion of PD was increased ($p<0.001$) with decreasing forage: concentrate ratio, with the highest rates of excretion of both allantoin and uric acid at the highest proportions of concentrate in the diet (Moorby *et al.*, 2006) [15].

Haematological profile

The data obtained from haematological studies was presented in Table 2 & 3. The mean values for WBC, lymphocyte, monocyte, granulocyte and hemoglobin of this study did not differ between groups on 42nd day and the results are in accordance with Mathivanan and Kalaiarasi (2007) [12], whereas mean values of RBC count were significantly increased at 42 d in CUD treated groups (T-2, T-3, T-4) compared to control.

Table 2: Mean values of WBC, lymphocyte, monocyte and granulocyte count of experimental groups

Treatment groups	Mean ± SE (n=6)	
	28 d	42d
WBC count (×10³/µl)		
T-1	237.25^{a}±7.87	234.57^{a}±5.81
T-2	239.17^{a}±8.11	231.67^{a}±2.69
T-3	238.33^{a}±8.15	230.85^{a}±4.70
T-4	237.67^{a}±8.10	229.43^{a}±7.85
LYMPHOCYTE count (×10³/µl)		
T-1	206.58^{a}±7.11	205.85^{a}±5.66
T-2	198.12^{a}±2.69	202.70^{a}±1.89
T-3	203.70^{a}±3.32	199.95^{a}±6.04
T-4	207.05^{a}±1.84	198.92^{a}±7.44
monocyte count (×10³/µl)		
T-1	7.43^{a}±0.46	6.27^{a}±0.29
T-2	8.25^{a}±0.21	6.53^{a}±0.31
T-3	8.08^{a}±0.27	7.15^{a}±0.41
T-4	9.18^{b}±0.21	7.35^{a}±0.35
Granulocyte count (×10³/µl)		
T-1	23.25^{a}±0.93	22.33^{a}±1.06
T-2	23.30^{a}±1.79	22.43^{a}±1.04
T-3	25.60^{a}±0.97	23.75^{a}±1.89
T-4	30.07^{b}±0.83	23.20^{a}±0.23

Means with different superscripts in each column differ significantly ($p<0.05$)

Table 3: Mean values of RBC count, hemoglobin, MCV, MCH and MCHC of experimental groups

Treatment groups	Mean ± SE (n=6)	
	28 d	42d
RBC count (×10⁶/µl)		
T-1	2.36^{a}±0.09	2.25^{a}±0.09
T-2	2.37^{a}±0.09	2.69^{b}±0.05
T-3	2.36^{a}±0.09	2.63^{b}±0.12
T-4	2.41^{a}±0.12	2.50^{b}±0.05
hemoglobin (g/dl)		
T-1	10.05^{a}±0.62	9.38^{a}±0.11
T-2	9.77^{a}±0.26	9.90^{a}±0.41
T-3	10.53^{a}±0.24	9.82^{a}±0.48
T-4	10.80^{a}±0.19	9.72^{a}±0.47
MCV(fl)		
T-1	105.28^{a}±1.75	93.52^{a}±0.92
T-2	114.98^{b}±0.57	107.17^{c}±0.79
T-3	114.57^{b}±1.15	105.75^{bc}±1.07
T-4	116.33^{b}±0.98	104.15^{b}±0.45
MCH(pg)		
T-1	33.08^{a}±1.08	23.95^{a}±0.69
T-2	40.72^{b}±0.64	36.73^{c}±1.00
T-3	40.47^{b}±0.47	36.35^{c}±0.46
T-4	42.55^{b}±0.73	32.93^{b}±0.85
MCHC (g/dl)		
T-1	31.42^{a}±0.60	25.65^{a}±0.63
T-2	35.47^{b}±0.40	34.37^{c}±0.67

T-3	35.37^{b}±0.38	34.42^{c}±0.21
T-4	36.58^{b}±0.50	31.67^{b}±0.67

Means with different superscripts in each column differ significantly ($p<0.05$)

According to Edwards *et al.* (1987) [4], one of the most important factors to be considered in reduction of TEC is the production of the hormone erythropoietin since it is the vital hormone that stimulates the bone marrow to create red blood cells. Talebi *et al.* (2005) [22] have showed that the number erythrocytes of animals in good health vary with diets and clinical conditions of the animal. Results of experiment on MCV, MCH and MCHC also revealed higher values for CUD treated groups as compared to control. The increase in the blood indices could be related to chemical composition of cow urine distillate.

Serum biochemical profile

Results obtained from serum biochemical studies were presented in Table 4 & 5. All the serum biochemical parameters showed no significant difference between the groups on 42nd day of experiment, but the average values for total protein on 42nd day were higher in CUD treated groups compared to control

The non significant difference in the serum protein in the present study is suggestive of normal metabolic process of liver parenchyma as the proteins are synthesized in liver tissue (Ahmad *et al.*, 2013) [1]. Similarly, triglyceride and total cholesterol levels were numerically lower on 42nd day in CUD treated groups as compared to control which could be an indication of its hypocholestrolemic effect. There was no significant difference in serum glucose level among different groups on 42nd day.

However, numerically lower glucose values were observed on 42nd day in groups supplemented with CUD prepared from native breed (T-2 and T-3). The lower circulatory glucose concentration in groups T-2 and T-3 was perhaps an indicative of increased turnover rate and utilization of glucose at tissue level (Tawfeek *et al.*, 2014) [23].

Table 4: Mean values of serum total protein, albumin, triglyceride and total cholesterol concentration of experimental groups

Treatment groups	Mean ± SE (n=6)	
	28 d	42d
total protein (g/dl)		
T-1	2.53^{a}±0.12	2.22^{a}±0.15
T-2	2.93^{b}±0.08	2.32^{a}±0.15
T-3	2.71^{ab}±0.06	2.33^{a}±0.23
T-4	3.23^{c}±0.12	2.25^{a}±0.20
albumin (g/dl)		
T-1	1.28^{a}±0.03	0.97^{a}±0.09
T-2	1.47^{b}±0.03	1.20^{a}±0.10
T-3	1.40^{ab}±0.05	0.97^{a}±0.15
T-4	1.45^{b}±0.06	1.15^{a}±0.08
triglyceride (mg/dl)		
T-1	128.17^{a}±11.85	142.50^{a}±12.21
T-2	120.50^{a}±2.17	137.33^{a}±9.52
T-3	136.17^{a}±7.59	138.33^{a}±12.77
T-4	142.33^{a}±8.81	139.50^{a}±5.50
total cholesterol (mg/dl)		
T-1	120.83^{a}±2.48	75.67^{a}±7.54
T-2	116.00^{a}±3.75	69.50^{a}±5.98
T-3	113.50^{a}±2.32	72.33^{a}±2.59
T-4	115.67^{a}±2.47	72.67^{a}±6.39

Means with different superscripts in each column differ significantly ($p<0.05$)

Table 5: Mean values of serum glucose, urea, creatinine and ALT of experimental groups.

Treatment groups	Mean ± SE (n=6)	
	28 d	42d
glucose (mg/dl)		
T-1	329.33^{a}±3.76	325.00^{a}±23.90
T-2	327.17^{a}±3.97	313.33^{a}±10.66
T-3	336.50^{a}±3.74	272.83^{a}±25.59
T-4	361.67^{b}±7.53	330.17^{a}±10.83
urea (mg/dl)		
T-1	6.65^{a}±0.70	4.98^{a}±0.25
T-2	5.25^{a}±0.44	5.58^{a}±0.42
T-3	6.40^{a}±0.79	7.02^{a}±1.04
T-4	6.72^{a}±0.22	5.60^{a}±1.48
creatinine (mg/dl)		
T-1	0.43^{b}±0.01	0.32^{a}±0.01
T-2	0.33^{a}±0.01	0.30^{a}±0.01
T-3	0.34^{a}±0.01	0.30^{a}±0.01
T-4	0.40^{b}±0.02	0.31^{a}±0.01
ALT (U/L)		
T-1	24.82^{a}±1.63	17.98^{a}±0.69
T-2	26.10^{a}±2.17	20.55^{a}±2.07
T-3	23.00^{a}±0.74	20.47^{a}±2.73
T-4	26.65^{a}±1.54	18.13^{a}±1.07

Means with different superscripts in each column differ significantly ($p<0.05$)

The results obtained in the present study were in conformity with the findings Mathivanan and Kalaiarasi (2007) [12] when conducted a study to examine the effect of Panchagavya and *Andrographis paniculata* as alternatives to antibiotic growth promoter and stated that serum total protein, albumin, globulin and glucose levels did not differ significantly between treatment groups.

Conclusion

Haematological and serum biochemical profile data obtained in this study revealed that CUD can safely be used without any harmful effects in broiler chicken when given at a dose of 10ml/liter of drinking water.

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