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Effect of native and cross breed cow urine distillate on performance of broilers

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Abstract
A study was conducted to compare the efficacy of native and crossbreed cow urine distillate (CUD) on performance of broilers during hot summer (May-June) using day old broiler chicks (n = 80) with twenty birds in each group. 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. Higher feed intake, body weights, body weight gain and lower feed conversion ratio was observed in groups given native breed cow urine distillate (T-2 and T-3) followed by T-4 (H.F crossbreed cow urine distillate) and T-1(Control). Overall, oxidative stress was decreased and antioxidant status was improved in group T-2 followed by groups T-3, T-4 and T-1. A 25% reduction of malondialdehyde (MDA) concentration in hemolysate on 42nd day in cow urine distillate treated groups was noted. Significantly higher superoxide dismutase (SOD) activity was observed on 42nd day in groups T-2 and T-3 (0.47 and 0.39 U/mg protein) compared to T-1 and T-4 (0.26 and 0.29 U/mg protein). The results of the experiment concluded that supplementation of cow urine distillate from Ongole and Sahiwal breed of cows was more efficient in reducing the oxidative stress and giving higher antioxidant effect compared to Holstein Friesian crossbred.

Keywords: Native breed, crossbred, cow urine distillate, broilers, growth

Introduction
Most of the antibiotic growth promoters act by modifying the intestinal flora of poultry, which are associated with poor health and decreased performance of birds (Bedford, 2000) [4] and has evoked efforts to find alternatives. Amount/availability of free radicals are controlled by systems called antioxidants which can reduce oxidation rate considerably and are synthesized in the body as well as supplied by dietary sources and nutraceuticals (Athavale *et al*., 2012) [2]. Recently, Council for Scientific Industrial Research (CSIR), India has identified cow urine distillate for its antimicrobial and antifungal properties (Mathivanan and Kalaiarasi, 2007) [9]. According to ancient literatures distillates of cow urine was one to be used mainly and the distillate was found to exhibit antioxidant effect (Krishnamurthi *et al*., 2004) [7]. Therapeutic values of cow urine including anti-hepatotoxic, anti-diabetic, anti-bacterial, immunomodulatory, wound healing, neuroprotective, geno-protective activities have been reported (Rachana and Sreepada, 2019) [14]. Experimentally, it has been proved that among the urine from various species, the urine of Indian cows is most effective (Banga *et al*., 2005) [3]. Therefore, the objective of the present investigation was to evaluate the comparative efficacy of native and crossbreed CUD administration on growth performance, antioxidant enzyme activity and immune response in broiler chickens.

Materials and Methods
Chemicals
All the chemicals used in the present study were of analytical grade and were obtained from Sisco Research Laboratories Pvt. Ltd., (Mumbai, India) and Himedia Laboratories Pvt. Ltd., (Mumbai, India).

Collection and preparation of 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℃ using glass distillation apparatus. The obtained CUD was stored at -40 C for further use.

Experimental animals, design and management

Eighty, day old broiler chicks belonging to single hatch were purchased from Balaji hatcheries, Chittoor and were randomly allotted into four experimental groups with 20 birds in each group. The chicks were allowed to acclimatize for a period of 4 days and 10ml of ketone free CUD was administered per liter of drinking water starting from 5th day to throughout the entire experimental period (42 days). Group T-1 served as control which received drinking water without CUD. Group T-2, T-3 and T-4 birds received drinking water mixed with CUD of Ongole, Sahiwal and H.F crossbred, respectively.

All the chicks were reared under deep litter system with uniform standard management practices throughout the experimental period. The birds were fed with starter ration for 4 weeks of age and latter with finisher ration ad libitum till 6th week.

Growth performance

The experimental period lasted 42 d. On weekly intervals (0th, 7th, 14th, 21st, 28th, 32nd and 42nd), birds were weighed and feed consumption was recorded. The FCR was calculated for each period.

Oxidative stress and anti-oxidant status

Six birds from each group were sacrificed on 28 and 42 d of experiment. Blood samples were collected from birds slaughtered on 28 d, whereas both blood and liver tissue samples were collected from the birds slaughtered on 42 d. Hemolysate and 10% tissue homogenate were prepared and used for analysis.

The concentration of MDA was measured by the method of Niehaus and Samuelsson (1968) [13], using thiobarbituric acid reactive substances by TCA-TBA method. The SOD activity was assessed by the procedure given by Misra and Fridovich (1972) [10]. Catalase activity was estimated according to Beers and Sizer (1952) [5] and GPx activity according to Rotruck *et al*. (1973) [15].

Immune response

Serum separated from the blood samples collected on 42 day of slaughter was used for immunological study. Humoral immune response was assessed by determining the ND-Haemagglutination inhibition (HI) antibody titers by HI test.

The HA unit of NCDV (R2B) was determined by using a standard protocol by using HI titer plate (Procedure A and B) of Allan and Gough (1974) [1].

Statistical analysis

Statistical comparisons of the results 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

Growth performance

The results of growth performance are presented in Tables 1, 2, 3 & 4.

The average total feed intake of different treatment groups in 42 days ranged between 3029.73g to 3413.48g. The feed intake of groups T-2, T-3 and T-4 was higher by 12.67, 6.73 and 0.47%, respectively.

The average body weight of the CUD fed groups were higher by 16.95, 10.72 and 2.12% in T-2, T-3 and T-4, respectively compared to control. Increased feed intake by groups T-2 and T-3 could be related to more body weight that need more feed consumption to supply their requirements (Saki *et al*., 2018) [16].

The feed conversion ratio of different treatment groups ranged from 1.50 to 1.61 with better FCR noticed in groups given Ongole and Sahiwal CUD (T-2 and T-3). The feed conversion ratio is always a very helpful benchmark to determine the profitability of a farm. The presence of antioxidants could partially interfere with oxidative protein denaturation, and would improve nutrient utilization and FCR (Seven, 2008) [17].

Results of present study are in accordance with Natarajan (2003) [12] who found that Panchagavya when mixed with poultry feed or drinking water @ 5ml/bird/day, increased the weight gain in broilers. Similar results were reported by Sumithra *et al*. (2011) [18] who reported that body weight gain and feed conversion ratio was better in Panchagavya treated group of White leghorn cockerels as compared to control.

Oxidative stress assay

Results of the present study showed significantly decreased MDA levels in both hemolysate and tissue homogenate of Ongole and Sahiwal CUD treated experimental groups (Table 5 & 6) on 42 day and it is in accordance with the results of Gosavi *et al*. (2011) [6] who observed a significant reduction in MDA values in gomutra ark treated group of rats as compared to control. Similar to the present findings, Lavania *et al*. (2011) [8] stated that cow urine reduced TBARS levels of liver and plasma in all experimental groups of rats in a dose dependent manner.

Table 1: Average weekly feed intake (g) per bird of experimental groups

Treatment	Weeks						Total
	1st week	2nd week	3rd week	4th week	5th week	6th week	
T1	66.74	220.04	385.65	590.00	790.12	977.18	3029.73
T2	70.43	222.17	420.00	610.00	890.88	1200.00	3413.48
T3	69.13	220.00	400.00	594.55	850.00	1100.00	3233.68
T4	67.39	221.61	390.00	595.00	790.00	980.15	3044.15

Table 2: Average weekly body weights (g) of experimental groups

Treatment	Weeks						
	0th day	1st week	2nd week	3rd week	4th week	5th week	6th week
T1	38.65	99.09	240.87	453.43	870.88	1230.29	1860.59
T2	38.30	105.35	255.43	500.39	990.53	1500.76	2176.00
T3	37.22	105.65	250.00	500.64	970.00	1412.76	2060.00
T4	37.52	102.61	243.13	490.00	890.15	1260.80	1900.00

Table 3: Weekly average body weight gain (g) of experimental groups

Treatment	Weeks						Total gain
	1st week	2nd week	3rd week	4th week	5th week	6th week	
T-1	60.44	141.78	212.56	417.45	359.41	630.30	1821.94
T-2	67.05	150.08	244.96	490.14	510.23	675.24	2137.70
T-3	68.43	144.35	250.64	469.36	442.76	647.24	2022.78
T-4	65.09	140.52	246.87	400.15	370.65	639.20	1862.48

Table 4: Average feed conversion ratio of experimental groups

Treatment	Weeks						Average
	1st week	2nd week	3rd week	4th week	5th week	6th week	
T-1	1.10	1.55	1.81	1.41	2.20	1.55	1.61
T-2	1.05	1.48	1.71	1.24	1.75	1.78	1.50
T-3	1.01	1.52	1.60	1.27	1.92	1.70	1.50
T-4	1.04	1.5 8	1.58	1.49	2.13	1.53	1.56

Table 5: Mean values of MDA, SOD, CAT and GPx in hemolysate of experimental groups

Treatment groups	Mean ± SE (n = 6)	
	28 d	42 d
MDA (µM/ml)		
T-1	0.14b ± 0.02	0.16b ± 0.007
T-2	0.06a ± 0.006	0.12a ± 0.009
T-3	0.08a ± 0.007	0.12a ± 0.007
T-4	0.09a ± 0.007	0.13ab ± 0.006
SOD (U/mg protein)		
T-1	0.99a ± 0.11	0.26a ± 0.05
T-2	1.24ab ± 0.13	0.47c ± 0.02
T-3	1.59b ± 0.10	0.39bc ± 0.04
T-4	1.25ab ± 0.18	0.29ab ± 0.03
CAT (U/mg protein)		
T-1	0.31a ± 0.04	0.31a ± 0.04
T-2	0.36a ± 0.07	0.45b ± 0.01
T-3	0.35a ± 0.05	0.38ab ± 0.02
T-4	0.30a ± 0.02	0.34a ± 0.02
GPx (U/mg protein)		
T-1	27.55a ± 2.48	19.16a ± 0.99
T-2	46.15b ± 2.09	21.63a ± 1.75
T-3	28.89a ± 2.49	20.60a ± 2.02
T-4	28.22a ± 1.41	20.01a ± 1.70

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

Antioxidant status

Results on antioxidant status were presented in Table 5 & 6. SOD activity in 42nd day hemolysate samples of different experimental groups ranged from 0.26 to 0.47 U/mg protein with groups T-2 and T-3 showing significantly higher values compared to control. The increased SOD activity indicates more defensive mechanism towards free radical damage to erythrocytes. SOD activity in liver tissue was found to be highest in groups given native breed CUD (T-2, T-3) followed by T-4 and T-1. The increased SOD activity might be attributed to its free radical scavenging property.

Erythrocyte and liver catalase activity was comparably higher in group supplemented with CUD of Ongole which could be attributed to the composition of CUD. The GPx activity in 28th day hemolysate samples of different treatment groups ranged from 27.55 to 46.15 U/mg protein with group T-2 being highest and control being lowest, whereas 42nd day hemolysate samples did not show any significant difference among the treatment groups compared to control. However, the GPx activity of treatment groups as compared to control was slightly higher. The GPx activity in liver tissue samples ranged from 76.13 to 90.60 U/mg protein with highest being recorded for T-2 followed by T-3. However, there was no significant difference between T-4 and T-1.

The results of the study are in agreement with the observations reported by Lavania *et al*. (2011) [8] who stated that cow urine inhibits lipid peroxidation and provides protection by strengthening the antioxidants like glutathione, superoxide dismutase and catalase. Similar results were also reported by Nagda and Bhatt (2014) [11] who observed that pretreatment with cow urine prior to lindane administration showed alleviation in the levels of SOD, catalase and GPx.

Immune status

Immunomodulation is the pharmacological stimulation of immune system which may augment or suppress the magnitude of immune responsiveness. A substance that alters the immune response by suppression (immunosuppressive) or enhancement is termed as immunomodulation.

Results obtained on HI antibody titer are presented in Table 7. They revealed that CUD given groups of the present study showed no significant difference when compared to control.

Table 6: Mean values of MDA, SOD, CAT and GPx in hepatic tissue of experimental groups

Treatment groups	Mean ± SE
	42 d
MDA (nm/mg protein)	
T-1	4.99b ± 0.34
T-2	3.26a ± 0.24
T-3	3.51a ± 0.10
T-4	3.54a ± 0.12
SOD (U/mg protein)	
T-1	7.19a ± 0.15
T-2	7.97b ± 0.19
T-3	7.95b ± 0.15
T-4	7.35ab ± 0.30
CAT (U/mg protein)	
T-1	2.52a ± 0.08
T-2	2.65a ± 0.08
T-3	2.48a ± 0.07
T-4	2.49a ± 0.07
GPx (U/mg protein)	
T-1	76.45a ± 0.81
T-2	90.60c ± 3.03
T-3	83.84b ± 1.25
T-4	76.13a ± 2.43

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

Table 7: Mean values of HI titer of experimental groups

Treatment groups	Mean ± SE (n = 6)
	HI titer (42 d)
T-1	2.01a ± 0.10
T-2	2.06a ± 0.18
T-3	1.96a ± 0.17
T-4	2.11a ± 0.13

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

Conclusion

Decreased oxidative stress and improved antioxidant activity was noticed in group T-2 (Ongole CUD), followed by group T-3 (Sahiwal CUD) which resulted in increased growth performance and higher feed efficiency in broilers given CUD (10ml/liter of water) of native cow breed. The improved antioxidant status and overall growth performance of T-4 (CUD from HF crossbred cows) compared to control was not impressive.

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