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Chemoprofiling of Punganur Cow Urine at Different Physiological Stages

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ABSTRACT

Cow urine is an important component of Panchagavya which contains plenty of biochemical constituents, vitamins, hormones, bioactive compounds and antioxidants. The present study was conducted to evaluate the biochemical changes in urine of Punganur cows. A total of 30 urine samples ten each from apparently healthy heifers, lactating and dry Punganur cows was collected. Qualitative analysis of Punganur cow urine at different physiological stages showed the absence of leukocytes, ketones, nitrogen, urobiliniogen, bilirubin, glucose, blood and Vitamin C. Quantitative analysis of minerals showed significantly higher values of Calcium, Magnesium and Phosphorous in urine of lactating cows when compared to dry cows and heifers. Whereas, urea, uric acid, protein and creatinine levels were found to be significantly higher in urine of dry cows followed by heifers and lactating cows. Urine protein to creatinine ratio (UPC) was more in lactating cows followed by heifers and dry cows which shows hyperbolic relationship with creatinine. The hormonal profile of urine showed significantly higher levels of cortisol and growth hormone (GH) in lactating cows, estrogen in heifers and thyroxine in dry cows. The level of Immunoglobulin G (IgG) and Epithelial growth factor (EGF) in urine of lactating cows was significantly lower when compared to heifers and dry cows. These results showed that physiological status had profound effect on biochemical composition of cow urine.

HIGHLIGHTS

- Importance of hormones in cow urine.
- Chemoprofiling and therapeutic importance of cow urine.

Keywords: Punganur cow, Minerals, Chemical constituents, Hormones, Bioactive compounds

Cow urine is an important component of Panchagavya since ancient times in India. Urine contains mainly water, minerals, urine casts and other excretory products of the body. Cow urine components are reported to have many beneficial effects. Urea, a product of protein catabolism acts as a strong antimicrobial agent, removes blood abnormalities and toxins, natural stimulant of urinary tract, activates kidneys and is diuretic (Achliya *et al*., 2004). Creatinine has antibacterial property, sodium purifies blood and checks hyperacidity, phosphorous helps in removal of stones from urinary tract, potassium acts as an appetizer and eliminates muscle fatigue, calcium acts as blood purifier, bone strengthener and germicidal, whereas uric acid act as diuretic agents and its antioxidant property correlates with its anticancer effect (Jain *et al*., 2010). Presence of copper and calcium promote its anti-obesity activity, skeletal and bone health (Randhawa, 2010). Thus,

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Cow urine having a balanced proximate composition of all the above compounds may restore their balance in the body and helps in curing the diseases.

Biochemical composition of urine was significantly altered in concert with the endocrine status, which are the clear indicators of physiological conditions of animals (Ramachandran *et al*., 2020). Thus the choice of urine in diagnosing endocrine disorders is gaining importance because of non-invasive sampling and better analytic sensitivity for hormones, many of which have short half-lives and rapidly cleared from the blood (Olooto, 2013). Monitoring urinary cortisol concentrations is a valid tool for studying and evaluating adrenal activity and acute stress in lactating cows (Morrow *et al*., 2000). Growth hormone enhances milk synthesis in high-yielding dairy cows.In recent years, a lot of interest has been generated among scientific community to develop or scientifically validate the Indigenous Technical Knowledge (ITK) as an alternate therapeutic or preventive approach using cow urine or its products. Despite the non-invasive sampling and the affordable price, urinalysis is still rarely used as a routine clinical diagnostic tool in farm animals. Urine has several advantages over saliva, serum and milk because it can be collected in large volume which enables for multiple assays (Collins *et al*., 1979). Even though there are various reports regarding the medicinal value of indigenous cow urine (Rachana and Sreepada, 2019), there exists lacunae regarding the composition of Punganur cow urine at different physiological stages which need to be established based on scientific approach. Hence, the present study was carried out on Chemoprofiling of Punganur cow urine at different physiological stages.

MATERIALS AND METHODS

Collection of urine samples

A total of 30 urine samples, each ten samples were collected during early morning hours in a sterile amber colored bottles from heifers, lactating and dry Punganur cows maintained at Livestock Research Station, Palamaneru, Chittoor, A.P. The collected samples were filtered using sterile cotton gauze to exclude the extraneous materials like hair, dung etc. The filtrate collected was allowed to pass through 0.2 µ filter syringe and filtrate was used for analysis. Study was carried out in Department of Veterinary Biochemistry,College of Veterinary Science, Tirupati.

Chemoprofiling of cow urine

Physical parameters

The physical parameters of urine such as colour, transparency, odour, foam etc were recorded by sensory evaluation. pH and specific gravity were determined using urine analyser.

Biochemical composition

Qualitative analysis of chemical composition for the presence of leukocytes, ketone bodies, nitrogen, urobilinogen, bilirubin, glucose, blood and vitamin C was done using the strip test method (URIT 50 wet Analyser) by briefly dipping a reagent strips into fresh urine. Biochemical constituents such as Calcium (0-Cressolphthalein method), Magnesium (Xylidyl Blue method), Phosphorus (Phosphomolybdate/UV method), Creatinine (Jaffe & enzymatic method), Protein urine (Pyrogallol Red method), Urea/BUN (Urease method) and Uric Acid (Uricase/peroxidase method) was evaluated using standard Biochemical kits purchased from Biosystems Diagnostics Pvt. Ltd, China using A15 Biochemical analyser.

Hormonal profile and bioactive compounds

Urine samples were centrifuged at 2000-3000 RPM for 20 min at 25° and supernatant fluid was used for analysis. The urine samples were analyzed immediately for Cortisol, Estrogen, Thyroxine, GH, IgG and EGF by using ELISA kits (Mekonnin *et al.,* 2017and Wai *et al.,* 2013).

STATISTICAL ANALYSIS

Results obtained were subjected to One Way ANOVA (Snedecor and Cochran, 1994) followed by Duncan´s multiple comparisons test (SPSS version 20). Significance was declared at $P<0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Physical parameters

Urine from heifers, lactating and dry Punganur cows was pale yellow in colour with clear transparency, having aromatic odour and produced foam on shaking. The cattle urine was normal in colour due to presence of urobilin (Reece, 2005). Similar observations were reported by the Kanujia and Upadhay (2018) where the fresh cow urine was pale yellow with light, strong and very strong odour. Present results are in concurrence with the earlier reports (Parrah *et al*., 2013) where the odour of cow urine as ammonical aromatic and shows clear transparency.

Table 1: pH and Specific gravity of Punganur cows urine at different Physiological stages

Parameter	Heifers	Lactating	Dry
Ph	$8.1^{a} \pm 0.10$	$8.1^{a} \pm 0.07$	$8.2^{a} \pm 0.08$
Specific gravity	$1.018^{a} \pm 0.01$	$1.018^{a} \pm 0.02$	$1.019^{a} \pm 0.01$

Values are mean ± SE (n=30); Means having same superscripts do not differ significantly (P>0.05).

The mean values of pH and specific gravity of urine for all the Punganur cows are presented in Table 1. No significant change in urinary pH and specific gravity was observed among the heifers, lactating and dry Punganur 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 and specific gravity obtained in this study are in agreement with Reece (2005) who reported the normal physiological range of pH as 7.0 to 8.4 and specific gravity as 1.015 to 1.040 for cattle. Similar results were obtained by Herman *et al*. (2019) in dairy cows and Ihedioha *et al*. (2019)in Nigerian trade cattle.

Biochemical composition

The chemical examination of heifer, lactating and dry Punganur cow urine revealed the absence of leukocytes, ketones, nitrogen, urobilinogen, bilirubin, glucose, blood and Vitamin C. The results are in accordance with the observations of Virshette *et al*. (2020) in Deoni cow urine. The surprising absence of an effect of lactation stage or proper nutrition on the ketone test strip results could be explained by the possible ruminal origin of ketone bodies as reported by Heitmann *et al*. (1987). The abnormal values of bilirubin, urobilinogen and ketones in the urine are not specific pointers of renal dysfunction, whereas glucose, erythrocytes, pH, specific gravity, nitrites and leucocyte levels are good indicators of renal function or presence of pathological condition in urogenital tract (Parrah *et al*., 2013).

Table 2: Levels of Calcium, Magnesium and Phosphorous in Punganur cow urine at different physiological stages

Minerals (mg/dl)	Heifers	Lactating	Dry
Calcium	$18.05^{b} \pm 0.34$	$22.32^{c} \pm 0.65$	$14.12^{a} \pm 0.41$
Magnesium	$8.80^{a} \pm 0.34$	$12.68^{b} \pm 0.61$	$8.12^{a} \pm 0.19$
Phosphorous	$3.11^{a} \pm 0.39$	$5.08^{b} \pm 0.29$	$2.91^{a} \pm 0.21$

Values are mean ± SE (n=30); Means with different superscripts in each row differ significantly ($p < 0.05$).

The mean values of calcium, magnesium and phosphorous were showed in Table 2. The quantitative analysis of minerals showed significantly higher levels of Calcium, Magnesium and phosphorous in urine of lactating cows as compared to that of heifers and dry cows. Cortisol induced increase in glomerular filtration of minerals may be responsible for their increase in urine as it is evident from increased urinary cortisol and minerals (Ca, Mg, P) during lactation. The results are in accordance with Filipejova *et al.* (2009) who reported significantly high values in lactating cows compared to dry cows. Increased phosphorous concentration in urine has also been observed in animals where saliva secretion is inhibited by feeding diets low in physical fiber (Knowlton and Herbein, 2002; Hill *et al.,* 2008).

Significantly high concentration of urea, uric acid, protein and creatinine were observed in urine of dry cows compared to heifers and lactating Punganur cows (Table 3). 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 the reason for high concentrations of urea and uric acid in dry cows compared to lactating cow and heifers. The present

findings were in accordance with Filipejova *et al*. (2009) who reported high concentration of urea during dry period compared to lactation in dairy cows. The observed mean protein values fell within the reference interval reported by Herman *et al*. (2019) for healthy cows, therefore all the animals included in the present study were non-proteinuric. Recovery of muscle mass after lactation may also have accounted for the higher creatininuria observed in the present study in dry cows compared to lactating Punganur cows. Steinhauslin and Wauters (1995) reported that UPC ratio measured in a spot urine sample can be used as a reliable estimate of the degree of proteinuria. In our study, lactating cows showed low urinary creatinine and high UPC ratio, which is in agreement with Herman *et al*. (2019) who reported hyperbolic relationship between urine creatinine concentration and UPC ratio.

Table 3: Biochemical constituents in Punganur cow urine at different physiological stages

Parameter (mg/dl)	Heifer	Lactating	Dry
Urea	$258.96^{a} \pm 10.81$	$234.17^{a} \pm 16.81$	$375.09^{b} \pm 22.91$
Uric acid	$24.11^{b} \pm 0.73$	$16.50^{a} \pm 0.81$	$34.89^{c} \pm 0.72$
Protein	$4.9^{b} \pm 1.87$	$3.6^{a} \pm 1.00$	$5.6^{c} \pm 2.73$
Creatinine	$32.23^{b} \pm 0.55$	$18.78^{a} \pm 0.93$	$48.69^{c} \pm 1.33$
UPC ratio	$0.15^{b} \pm 0.018$	$0.19^{c} \pm 0.003$	$0.11^{a} \pm 0.007$

Values are mean ± SE (n=30); Means with different superscripts in each row differ significantly ($p < 0.05$).

Hormones

Cortisol, estrogen, thyroxine and growth hormone levels were showed in Table 4

Cortisol

Cortisol levels were significantly high in urine of lactating cows followed by drycows and heifers which may be due to the stress, caused by milking during lactation. Urinary cortisol was used as an indicator of stress instead of plasma cortisol because of its non-invasive sampling procedure (Higashiyama *et al*., 2005). According to Morrow *et a*l. (2000), monitoring urinary corticosteroid concentrations is a valid tool for studying and evaluating adrenal activity and acute stress in lactating dairy cows.

Estrogen

Punganur cows showed significantly higher estrogen values in urine of heifers followed by dry and lactating cows. Similar results were reported by Naik *et al*. (2013) in serum of Punganur cattle during estrus. Mekonnin *et al*. (2017) reported that pregnant heifers in term trimester had significantly higher estradiol levels than heifers in-heat and in other reproductive states. The availability of steroid metabolite in higher concentration in urine (by 2-to 3-fold or more) than that of parent sex steroids in blood have given greater advantage to the use of urine in domestic and wild animals (Yang *et al*., 2004).

The results of this study could be helpful in determining physiological urinary steroid levels in order to provide a baseline for the control of steroid abuse in beef production (Snoj *et al*., 2014) and also enables the detection of cycling animals and hence to successfully breed them, and to perform pregnancy diagnosis as early as 18-days post service.

Thyroxine

Thyroxine levels were low in urine of lactating cows and heifers compared to dry cows. Our results are in agreement with findings of several authors (Huszenicza *et al*., 2001; Sinka *et al*., 2008 and Stojic *et al*., 2001) who reported that decreased levels of thyroid hormones particularly in early lactation may be due to the mobilization of body reserves for high milk production. Paulikova *et al*. (2011) reported that high concentrations of thyroid hormones observed in dry cows decreased significantly in peri-partal period in accordance with their nutritional and metabolic processes. Low levels of thyroid hormones in early lactation may be one of the dairy cows mechanism of reducing metabolic demand as reported by Tiirats (1997).

Growth Hormone

The positive correlation was observed by Walker *et al*. (1990) between peak serum response to provocation and overnight urinary growth hormone excretion and confirmed that urine test may be as good as blood test. Results showed high urinary GH concentration in lactating cows compared to heifers and dry cows which are in accordance with Kirovski *et al*. (2012) who reported an increase in GH and low levels of insulin to facilitate adipose tissue

mobilization in support of milk production during early lactation. As it was evident that GH in cow affect the release of urea from liver and increased deposition of protein in liver (Bell *et al*., 2000). It may be the reason for low levels of urea and protein and high concentration of GH in lactating cows.

Table 4: Hormonal profile in Punganur cow urine at different physiological stages

Parameter	Heifers	Lactating	Dry
Cortisol (ng/ml)	17.17^{a}±0.15	26.05^{c}±1.03	20.95^{b}±0.47
Estrogen (ng/L)	31.30^{c}±0.22	27.73^{a}±0.27	30.15^{b}±0.42
Thyroxine (ng/ml)	37.11^{a}±0.64	42.71^{b}±0.80	44.60^{b}±0.75
Growthhormone (ng/ml)	41.34^{b}±0.81	42.27^{b}±0.30	36.86^{a}±1.78

Values are mean ± SE (n=30); Means with different superscripts in each row differ significantly ($p < 0.05$).

Bioactive compounds

IgG

Low levels of IgG was observed in urine of lactating cows compared to dry cows and heifers (Table 5). The present findings are in coincidence with Singer *et al*. (2015) who reported that increase in systemic oxidative stress is associated with a generalized decrease of serum IgG antibody responses to the periodontitis. As reported by Sawangsoda *et al*. (2012), the physiological concentration of IgG in urine is 10,000 times less than that in plasma, the detection of antibodies in urine have been suggested as the possible alternatives for plasma/serum for the diagnosis of various parasitic diseases because of less invasiveness of the collection of urine samples. A significant correlation between antibody levels in serum and in urine has been reported in the diagnosis of *Schistosoma japonica* and hepatitis-A (Itoh *et al*., 2003; Joshi *et al*., 2002) and strongyloidiasis (Eamudomkarn *et al*., 2018).

Epithelial growth factor

Epithelial growth factor (EGF) acts as potential stimulator of bladder epithelial cell replication, formerly known as urogastrone, because of its presence in high concentrations particularly in the urine (Jorgenson *et al*., 1993). Increase in urinary EGF levels was observed in dry cows compared to heifers and lactating cows (Table 5). The values of urinary EGF obtained in our study are in agreement with Camli *et al*. (2005) in rat urine (0.53-1.33 ng/ml) and Zhang *et al*. (2003) in human urine (1.8-2.7 ng/ml). As reported by Kwon *et al*.(2010), it can be used as a biomarker for diagnosis of acute kidney injury.

Table 5: IgG and EGF in Punganur cow urine at different physiological stages

Parameter	Heifers	Lactating	Dry
IgG (µg/ml)	57.68^{b}±0.78	47.15^{a}±0.77	63.66^{c}±1.03
EGF (ng/ml)	1.16^{a}±0.00	1.17^{a}±0.00	1.22^{b}±0.02

Values are mean ± SE (n=30); Means with different superscripts in each row differ significantly ($p < 0.05$).

CONCLUSION

It may be concluded that altered biochemical constituents in urine samples of Punganur cow may be due to nutritional and metabolic demands during different physiological stages that require constant adjustment of several mechanisms to regulate the metabolism of dairy cattle. The data generated on hormone analysis in urine samples of Punganur cow at different physiological stages may be useful as a reference for further research.

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