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Analysis And Versatile Uses Of Indian Cow Urine A Review. ...

Dr. Gudabandi Vijaya Pratap[1]*, Dr. Bellala Krishna[2], Dr. Tulasi Bhushan Rao[3], V. Tejeswara Rao[4*]

[1*&3]Asst. Professor of Zoology, Dr.V.S.Krishna Government Degree & PG College (A). Visakhapatnam, Andhra Pradesh-530013
[2]Asst. Professor of Zoology, Government Degree College (W), Marripalem, Alluri Sitaramaraju District, Andhra Pradesh.
[4*]Associate professor, MVR Degree College (UG & PG Courses), Gajuwaka, Visakhapatnam-530026

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Abstract

In Ayurveda, cow urine (Gomutra) occupies a unique place and has been recognized as water of life or "Amrita". Urine collection is one of the most basic procedures in animal experiments such as hormone trials and nitrogen balance studies. Because of the complicated structure of the urinary anatomy, there has previously been no perfect apparatus and method to collect urine continuously and precisely from cows and heifers without having negative effects on their health and performance. This is also one of the reasons that bulls and steers are used as replacements in many experiments. Scientific results may not be applicable because of the huge differences in the aspects of physiological structure, nutrient requirements, and metabolic characteristics between cows.

Keywords: Cow urine, Urine cup, urine collection, apparatus, method

Introduction

Ayurvedic texts (Sushruta Samhita, Ashtanga Sangrah and Bhav Prakash Nighantu) describe cow urine (CU) (gomutra) as an effective medicinal substance/secretion of animal origin with innumerable therapeutic uses. Cow (Kamadhenu) has been considered as a sacred animal in India. In Rigveda (10/15), CU is compared to nectar. In Susruta (45/221) and in Charak (sloka-100) several medicinal properties of CU have been mentioned such as weight loss, reversal of certain cardiac and renal diseases, indigestion, stomach ache, diarrhea, edema, jaundice, anemia, hemorrhoids and skin diseases including vitiligo. Gomutra is capable of removing all the imbalances in the body, thus maintaining the general health [1]. CU contains 95% water, 2.5% urea, minerals, 24 types of salts, hormones, and 2.5% enzymes. It also contains iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, iron, sulfur, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokine and lactose [2].
An apparatus and method that can allow continuous and precise total collection of urine under normal conditions was developed that could satisfy the urine sampling requirement of balance studies, metabolic experiments, and hormone trials of cows.
In the past, manual collection of urine has been used, with great labor cost. This has prevented cows from being fed in a normal environment and has probably affected the reference for urine volume. Described an apparatus in which a rubber tube was strapped over the vulva by using a harness transfer for conducting the urine to a container. Improved the above apparatus by using branding cement to attach the straps and tube to the cows. The 2 similar apparatuses could collect urine effectively, but removal of the devices was rather difficult and caused raw spots devoid of hair where the straps were fastened with branding cement. Furthermore, during the standing up and lying down activities of cows, there was a potential risk of leaking urine because the apparatus did not have a totally closed urine collection tube. Modified the apparatus by using a harness with a feces-collection bag and a rubber tube to conduct urine into a container placed on the floor. Reported an apparatus that collected urine and feces concurrently, which provided mobility by using a portable urine bag and a smaller, lighter weight feces bag based on the model. A urine cup for long-term collection of urine from cows, using glue to place straps in position, was described by Fellner [3-7]. However, the apparatus required frequent supervision and rearrangement because the device and feces weighed too much. The method gave good results only with some cows. Additionally, it was easier

to collect feces than urine. Although the different methods described above had many advantages, there were also some major disadvantages, such as the complexity of the apparatus, the difficulty of handling procedures, and the negative effects on cows. The objective of this paper is to describe an easy-to-handle method and an inexpensive, portable, and reusable apparatus to collect urine continuously and precisely, with few negative effects on the cows.
Cow's urine samples from local cows, were collected early morning at around 5am when cows micturated first time in the day, in sterilized containers. Compared to a sample taken in noon this would prove more effective.
Note: For this research, urine extracts from local cows were taken.
Cow urine sample is to be collected from the Goshala, which maintains forty different breeds of cow. The cow to be selected for our research work will be a healthy Gir cow aged seven years having a uniform diet and undergoing regular vaccination schedule.
The fresh cow urine will be collected in sterile screw capped bottles and brought to the laboratory for testing. Fresh cow urine can be subjected to testing after filtration by ordinary filter paper.
Sterile cow urine can be prepared by sterilizing the urine sample in an autoclave at 121°C temperature and 15 lb/in2 pressure for 15 minutes.
Photo activated cow urine can be prepared by keeping the urine in transparent sterile bottle for 144 hours in sunlight. Then it can be purified on a silica gel G-25 column and passed through two separate columns simultaneously to get rid of all the precipitated material and debris.
Cow urine distillate can be obtained by subjecting the fresh urine sample to a distillation unit where the distillate can be obtained after 4 – 5 hours of distillation. Different samples of cow urine like
· Fresh cow urine,
· Sterile cow urine,
· Photo activated cow urine,
· Cow urine distillate,

These are subjected to antimicrobial activity by agar diffusion technique, using the most common human pathogenic bacteria and fungi., viz.
These cultures are
· Escherichia coli
· Bacillus subtilis
· Staphylococcus aureus
· Proteus vulgaris
· Salmonella typhimurium
· Aspergillus fumigates
· Candida albicans

Method:-

1. The plates will be inoculated by dipping a sterile swab into inoculums culture.
2. The swab will be streaked all over the surface of the medium. The inoculums will be dried for a few minutes at room temperature with the lid closed.
3. The sensitivity testing discs or cups will be placed on the inoculated plates using a pair of forceps.
4. The cups or discs will be saturated with known amount of urine sample using a micro pipette.
5. The sample will be allowed to diffuse into the agar medium.
6. The plates will be incubated at 37°C for 24 – 48 hrs.
7. The diameter of the zone of inhibition will be measured in m.m.

Urine Cup

The urine cup was composed of an infundibular rubber cup, canvas, drainage hose, and straps. Bicycle or car tire inner tubing of 0.2-cm thickness, attached to the distal end with rubber cement, could be used as the primary material for the infundibular rubber cup. According to the shape of the infundibular rubber cup, canvas, with a thickness of 0.2-cm, was cut to dimensions large enough to cover the vulva areas of the cows and was sewn onto the infundibular rubber cup with plastic thread. At the same time, 8 circular holes were made in the canvas to fix the

straps onto the cows. The straps were made of flexible rubber hose with a 1.0-cm inside diameter and a 0.2-cm thickness, which could ensure that the pressure was suitably distributed and that there were no injuries to the body, especially to the mammary vein. The large upper diameter of the urine cup was approximately 40 cm, which should be a suitable size to cover the edge of the buttocks area entirely. The small lower diameter of the urine cup was about 3.0 cm, and this must be in close contact with the drainage hose. A 3.0-cm diameter drainage hose made of transparent polyvinyl chloride flexible pipe was then fixed onto the small lower end of the urine cup by means of steel wires, to conduct the urine from the urine cup to the collection barrel. Suitable cleanness with detergent and rinsing in hot water after each collection period would ensure that the urine cup was reusable many times.

Collection Barrel

A barrel made of transparent hard plastic would be a good choice as a collection barrel. The diameter of the collection barrel opening should be approximately 3.5 cm to ensure that the drainage hose can be inserted into the barrel. The urine was removed and collected from the barrel twice each day in most of the experiments; therefore, the volume of the collection barrel should be about 20 L because cows can produce 9.1 to 35.7 L/d of urine.

Preparation Methods

Preparation of Cow's urine sample:
Fresh urine samples were collected from the cattle breed in Palakkad district of Kerala, and Bangalore, Karnataka, and refrigerated in closed, air-tight, sterilized containers, to prevent oxidation. These samples were filtered and cleared from any other particulates before being used as the test material.
Note: It was ensured that the urine sample collected was the first urine micturated early morning once the cattle were awakened.
Preparation of Micro-organism cultures:
For preparing the Sclerotinia and Penicillium cultures, the MTCC culture samples for both were pure-cultured and sub-cultured to obtain pure strains of Sclerotinia sclerotiorum and Penicillium expansum cultures. Sub-cultures were prepared using techniques of both streak and slant cultures. Escherichia coli DH5 Alpha cells were pure-cultured using Luria Bertani Medium (LB) and then sub-cultured.
Nutrient Agar and Potato Dextrose Agar (PDA) was used as the media for bacterial and fungal cultures, respectively, for all the tests using urine.

Tests

Anti-Bacterial/Anti-Fungal Tests
The main techniques used for the anti-bacterial/anti-fungal tests were – the pour-plate test (for both bacteria and fungi) and the well-plate test (for bacteria).
In the pour-plate technique for anti-fungal test, 500µl/1000µl of cow's urine sample was pour-plated with the PDA mixed with a pinch of ampicillin, in autoclaved petridishes. These plates were then spread with the spores of Sclerotinia sclerotiorum and Penicillium expansum from pure cultures, and kept aside undisturbed for 4-5 days to observe the fungal growth pattern.
For anti-bacterial test, 1ml of cow's urine sample was pour-plated with the nutrient agar, in autoclaved petridishes. A fine superficial spread of DH5 Alpha bacteria from pure cultures was made on these plates and kept aside undisturbed for 24 hours to observe the bacterial growth pattern.
In the well-plate technique for anti-bacterial test, 100µl of cow's urine sample was micropipetted into wells within the Nutrient agar, in autoclaved petridishes. These plates had already been pre-spread with a fine superficial layer of DH5 Alpha bacteria from pure cultures. The plates were kept aside undisturbed for 24 hours to observe the bacterial growth pattern.

Thin Layer Chromatography (TLC) Analysis of Cow's Urine for Enzyme Detection
Silica Gel slides were prepared by introducing silica gel of thick sloth-like consistency, on clean slides and leaving the slides undisturbed for solidification. Cow's urine sample drop was introduced (spotted) on one edge of each of the slides, and then these slides were slightly immersed in the running solvent comprising chloroform to acetic acid

in the proportion 8:2.[8]. [9]. The slides were left undisturbed for 30-45 min, and then sprayed with ninhydrin solution on the spotted areas within each slide, and left in hot air oven for drying. The pink color observed on the spotted areas indicated presence of proteins, which confirmed the presence of an enzyme.
Researcher [10] shows cattle urine contains nitrogen, sulphur, ammonia, copper, phosphate, sodium, potassium, manganese, carbolic acid, iron, uric acid, urea, silicon, chlorine, magnesium, calcium, lactose, enzymes, creatinine, aurum hydroxide .Total N in the cattle urine ranged from 6.8 to 21.6 g N litre−1 , of which an average of 69% was present as urea, 7.3% as allantoin, 5.8% as hippuric acid, 3.7% as creatinine, 2.5% as creatine, 1.3% as uric acid, 0.5% as xanthine plus hypoxanthine, 1.3% as free amino acid N and 2.8% as ammonia [11].
In the present study we have used flame AAS for the determination of Cu, Ca, Zn and Fe in the urine of normal cycling healthy cows.
Collection of urine Midstream early morning urine samples were collected from indigenous cattle's. Total 20 samples were collected in wide mouth plastic bottles. Due care was taken to avoid contamination in the sample. Therefore, the bottles were washed with 1% nitric acid. Samples were stored at -20^{0}C until analyzed.

Reagents Used:

All reagents used for this work were of analytical grade and they include: nitric acid (HNO_3), perchloric acid ($HClO_4$) and sulphuric acid (H_2SO_4). Solutions were prepared using doubly-distilled deionised water.

Preparation of reagents:

Triple acid mixture Concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$) and sulphuric acid (H_2SO_4) were mixed in the proportion of 3:3:1.

Estimation of trace elements:

Estimation of trace elements (Cu, Ca, Zn and Fe) in urine samples of cow was done using flame mode of Atomic Absorption Spectrophotometer (Model No. AAS 400; Perkin Elmer, USA). Procedure For analysis, samples were brought to normal temperature. Samples were digested by tri-acid mixer (HNO_3:H_2SO_4: $HClO_4$ = 3:1:1). 20 ml sample was mixed with 15 ml tri-acid mixer. They were digested at 250^{0}C until a clear digested solution was obtained. The final volume was made to 40 ml and filtered through Whatman filter paper 42 to remove silica and other insoluble residues from digested samples.
A series of working standards were run in AAS for the preparation of the standard curve. Finally, the samples were run and the readings were noted. Concentrations of minerals were estimated by multiplying AAS value with dilution factor.
Blank Determination Blank determination was carried out following the same procedure described above for urine samples. 0.1 M HNO_3 in the final solution maintained the acidic environment and avoided formation of insoluble hydroxides before analysis. The digested blank samples were analysed for Cu, Ca, Zn and Fe.

Salient Features of Cow Urine:

- An approach towards sustainability.
- Expense-free farming.
- Producing quality and poison-free food.
- Agriculture without external input.
- Farming in tune with nature.
- Protecting soil fertility and soil health.
- Maintaining the level of organic matter.
- Encouraging biological activity in soil.
- Providing nutrients through the microbial action.

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