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Physical and microbial characteristics of fresh urine and dung of heifer and lactating Sahiwal cow

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Abstract
The present study was undertaken with the objective to study the Physical and microbial characterization of fresh urine and dung of indigenous (Sahiwal) cow. The indigenous cows (Sahiwal) present at Livestock Research Centre, National Dairy Research Institute, Karnal was selected for urine and dung collection for bio-chemical estimation. The results of the present study revealed that most of the physical parameters of lactating cows and heifers fresh dung and urine were found similar and differed non-significantly except, pH content of urine which was found significantly (P<0.01) higher in lactating Sahiwal cows as compared to the heifers. Whereas, the Total bacterial count (TBC) and Total Yeast and Mould count (TYMC) was found significantly higher in cow dung and urine as compared to heifers.

Keywords: Urine, dung, Shiwal, Cow

Introduction
Cow urine has many beneficial properties particularly in the area of agriculture and therapeutics. It has also been observed in scientific research that the urine of Indian cows is highly effective as compare to the urine of other species. Cow urine is a good bio-pesticide and also effective against many diseases including cancer and is a very potent immune enhancer. In Sushruta Samhita and Ashtanga Sangraha, cow urine has been described as the most effective substance/secretion of animal origin with innumerable therapeutic values (Dhama *et al*., 2005) [7]. Cow urine contains 95% water, 2.5% urea, and 2.5% minerals, salts, hormones and enzymes. It contains iron, calcium, phosphorus, salts, carbonic acid, potash, nitrogen, ammonia, manganese, sulphor, phosphate, potassium, urea, uric acid, amino acids, enzymes, cytokines, lactose etc. Cytokines and amino acids present in urine may play a role in immune-enhancement (Bhadauria, 2002) [5].
Cow urine is one of the components of popular preparations such as Panchagavya, Amrutpani and Jiwamrut used as biofertilizers and for composting farm waste (Dhama *et al*., 2005; Salkinkop *et al*., 2005) [7, 18]. Experts also suggest it to be used for spraying as an organic fungicide or pesticide. After regular use of cow urine in the crops it is found that soil microorganisms has increased along with the crop production. Cow urine works as plant growth promoter. Cow urine is used by the farmers as an effective indigenous method to control crop pests (Banjo *et al*., 2003) [4] and spraying of the cow urine has been recommended to minimize the harmful effects of synthetic pesticides (Chauhan and Singhal, 2006) [6]. For this purpose, stored/fermented stock is preferred over fresh urine because it can cause leaf scorching or burning and plant wilting due to hippuric acid and urea present in cow urine (Peterson *et al*., 2012) [16].
Cow urine can be diluted up to 10% with water and applied to the crop (Shete, 2016) [21]. Cow dung and cow urine enhances the insecticidal activity of panchagavya which can reduce the number of application hazardous chemicals on crops (Shailaja *et al*., 2014) [19]. It has been shown that cow urine extract of certain plants as well as cow urine in combination with certain plant extracts are found to possess marked inhibitory effect on human pathogens as well as plant pathogens (Akhter *et al*., 2006; Yadav *et al*., 2008; Rajapandiyan *et al*., 2011; Tiwari & Das, 2011) [2, 24, 17, 23].
Ananda (2011) [3] characterization of total microbial and fungi population in cow urine and revealed the total bacterial population was $260x10^4$cfu/ml which shows highest population while the fungi was $0.04x10^4$cfu/ml. The value represents average of three replications. Elemam (2003) [9] studied two urine samples A and B collected from a milking cow and heifer respectively were examined. The milking cow urine was clear yellow with pH 7.64 and the heifer urine was slightly yellow with pH 7.63 (Plate 2).

The milking cow urine was found to have high microbial load (4.3×103 cfu/ml) than the heifer urine (3.1×102 cfu/ml). Elbashir *et al.* (2004) [8] reported that the camel and cattle urines were alkaline with an average of 9.5, 8.6, respectively while the goat and human urine are acidic with average of pH 4.85, 6.5 respectively. This variation in pH was attributed to feed intake (high energy diets), such diets can cause metabolic acidosis, mineral distribution and environmental conditions. The initial pH in compost heaps is generally slightly acidic, around 6 pH is found in the cell sap of most of the plants. The production of organic acids during the early stages of composting causes further acidification (pH 4.5-5.0) whenever the temperature rises.

Kiyasudeen *et al.*, (2015) [11] studied yeast and mould loads of the three cow dung samples were expressed as colony forming units (cfu/g).total CFU/g of fungi in CD3 (2.78±0.01x10 cfu/g) is substantially higher than CD2 (2.36±0.04 x10 cfu/g) and CD1 (2.14±0.01 x10 cfu/g). Mangalanayaki and Thamizhmarai, (2016) [13] Studies the microbial load of total bacteria in cow dung. The maximum number of their microbial population was exhibited in dilution 10^4 which ranged from 55.5 $\times 10^4$ to 190.4$\times 10^4$ cfu/ml and minimum concentration was exhibited in dilution 10^6 which ranged from 20.0$\times 10^6$ to 53.6$\times 10^6$.

Materials and Methods

Collection of cow urine and dung

Fresh Cow urine and dung was obtained from Livestock Research Centre, Karnal, early in the morning from healthy indigenous heifer and lactating Sahiwal cows.

Characterization of cow urine:

After wiping away faecal matter from the vulva, the cows was stimulated to urinate by stroking the side of the vulva for approximately 15-30 seconds and approximately 50 ml of mid-stream urine was collected from each animal in clean containers and then transferred into sterile labeled bottles and stored in refrigerator for further analysis. The pH of fresh urine was read directly using pH meter (Hanna pH 210 Microprocessor pH meter). The total microbial and yeast and mould population from fresh cow dung and cow urine were analyzed by serial dilution technique and plated in suitable media.

Estimation of total microorganisms in cow dung, urine and different formulations

The total microbial and yeast and mould population from fresh cow dung, cow urine and different formulations were analyzed by serial dilution technique and plated in suitable media.

Plate count agar

The medium was composed of tryptone, yeast extract, D-glucose and granulated agar. It was prepared according to manufacturer's instructions by using 23.5g in 1 liter distilled water. The medium was allowed to boil in water bath until it was completely dissolved. The pH was adjusted to 7.0, and then the medium was sterilized in an autoclave at 121 ºC for 20 minutes. The medium was used for counting of microflora.

Potato Dextrose Agar

Suspend 39 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before dispensing. In specific work, when pH 3.5 is required, acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile, cooled medium is approximately 1 ml. Do not heat the medium after addition of the acid.

Serial dilution technique

Bacteriological examination and enumeration was done for formulations at different days of fermentation ie, 0^{th}, 10^{th}, 20^{th} and 30^{th} days. One ml of sample was mixed with 9 ml of sterilized distilled water to get 10^{-1} dilution, transferred 1ml of 10^{-1} to 9 ml sterilized water blank to get 10^{-2} dilution were continued till 10^{-6} to 10^{-8} dilution. Transferred 1 ml aliquot from required dilutions to petriplates, 10^{-1} and 10^{-2} dilutions were used for yeast and fungi and 10^{-6} and 10^{-8} dilutions were used for bacteria. The melted and cooled media was transferred to respective prelabeled plates like nutrient agar media to bacterial plates, Potato dextrose agar media to fungi plates. After plating, kept the plates for incubation for about 24 to 48 hours for bacteria and 48-72 hrs or sometimes 3 to 4 days for fungi at temperature of 28^0 C. The same procedure was adopted for urine analysis except different day's analysis.

Preparation of cow dung suspension

Cow dung suspensions were prepared by serial dilution method. The collected and labeled, 1gm of cow dung samples were mixed in 10 ml sterilized phosphate buffer and vigorously shaked in vortex for 2 minutes for proper mixing of sample. Before plating, all the samples were incubated at 37 ^{0}C for 30-40 minute in an incubator for activation of microorganism. After incubation dilutions of each sample were prepared by using standard dilution method with the help of sterilized pipette. In this method, Phosphate blanks were prepared, each contain 9 ml of sterilized phosphate buffer. The labeled tubes were placed in test tube stand then 1ml of activated standard solution was transferred aseptically in test tube number 1, and further 1ml of sample was transferred to number 2 and same procedure was repeated for each dilution after that incubated at room temperature for 24 hrs. Plates showing well separated, isolated bacterial colonies were taken for further analysis. In case of fungus, colonies were counted after 48-72 hrs.

Results and Discussion

Physical property of fresh urine and dung of heifer and lactating cow

pH in lactating cow and heifer urine

The pH content of heifer and lactating cow in the experiment has been presented in Table Urine samples collected from a milking cow and heifer respectively were examined. The urine of heifer was yellowish brown with pH 7.70±0.12 and the milking cow urine was dark yellow with pH 8.63±0.13 and was significantly higher (P<0.01) than the heifer urine. Similar value was reported by Elbashir *et al.* (2004) [8] who found that cattle urine was alkaline, Mavangira *et al.* (2012) [14] who reported fresh cow urine on the alkaline side, Sonthi (2010) [22] who also reported the pH of fresh cow urine is in alkaline nature.

pH in lactating cow and heifer dung

The pH of heifer and lactating cow dung of experimental animal is presented in Table 4.1. The pH of heifer and cow dung was 7.24±0.17 and 8.19±0.18 respectively. The milking cow dung was found to have significantly (P<0.05)) highest pH value than the heifer dung. Present values were higher than those reported by Adesanya *et al.* (2017) [1] and

Godambe and Fulekar (2016) [10] were investigated Sahiwal and all lactating phase cows' dung. The total pH was more in the urine than in dung.

Dry matter (%) in cow and heifer dung

Table 4.1 shows that total solids or dry matter % content is an important factor in determining the handling characteristics and relative nutrient content of manure. The levels of total dry matter % of cow dung and heifer were 18.49±0.58 and 19.51±0.57 respectively. There was no significance difference (P>0.05) of dry matter % between cow and heifer dung. Present values were higher than those reported by Kumari (2007) [12] 16.2% and Miner *at el.,* (1975) [15] who found 15% dry matter in lactating cow. Similar values were reported by Adesanya *et al.* (2017) [1] studied on characterization of dairy farm manure and reported that the dry Matter (%) content for the lactating and dairy cow was 19.71and 19.02 respectively while the heifer had 24.19% dry matter which was higher than the present findings.

Moisture (%) in cow and heifer dung

The moisture level of present finding is shown in table 4.1. Significantly higher (p<0.01) moisture level was observed in lactating cow dung (81.51±0.27) compared to heifer (80.49±0.20). Moisture is one of the major parameter in estimating the quality of any manure or substrate. Similar values were reported by Adesanya *et al.* (2017) [1] studied on characterization of dairy farm manure and reported that the Moisture (%) content for the lactating (80.29) and dairy cow (80.98), while dry matter % of Heifer dung was reported 75.81% which was lower than present findings.

Microbial population in cow dung and cow urine

Microbial population in lactating cow urine

The microbial population in cow urine is presented in Table 4.2. The total bacterial population in heifer and cow were 8.83±0.46 and 12.00±0.81 (10^8cfu/ml) respectively, while the yeast and fungi 3.59±0.4 and 5.12±0.49 (10^3cfu/ml) respectively. The milking cow urine was found to have higher microbial load than the heifer urine. The total microbial load was more in the dung than in urine. The values of microbial population found in the present study was lower than those reported by Ananda (2011) [3] who obtained total bacterial population of $260x10^4$ cfu/ml, while the fungi was $0.04x10^4$ cfu/ml which was lower than the present finding. The present values of microbial population obtained present study was higher than those reported by Elemam (2003) [9] who reported milking cow urine to have high microbial load (4.3×10^3 cfu/ml) than the heifer urine (3.1×10^2 cfu/ml)

Microbial population in cow dung

The microbial population in cow dung is documented in Table 4.2. The total bacterial population in heifer and lactating cow was 16.17±0.7 and 20.16±1.58 cfu/g respectively. The yeast and fungi were (5.46±0.43 and 7.73±0.47cfu/g) respectively. It was significantly (P<0.05) higher population of microorganism in lactatin cow dung as compare to heifer. Present finding are not in agreement with of Kiyasudeen *et al.* (2015) [11] who reported total viable counts of bacteria to be (1.78±0.05 to 2.84±0.01x0^5). However values highest than the present finding have been reported by Mangalanayaki and Thamizhmarai (2016) [13] in dilution of 10^4 which ranged from 55.5 $\times10^4$ to 190.4×10^4 cfu/ml and in dilution of 10^6 ranging from 20.0×10^6 to 53.6×10^6. Sharma and Singh (2015) [20] reported that the maximum number of bacterial population was exhibited in dilution of 10^4 which ranging $60.5x10^4$ to$175x10^4$ cfu/ml and in dilution of 10^6 which ranged from $23.5x10^6$ to $80.5x10^6$. The values of total yeast and fungi of present finding were higher than the values reported by Kiyasudeen *et al.* (2015) [11] (2.14±0.01 x10 to 2.78±0.01x10 cfu/g).

Table 1: Physical property of fresh urine and dung of heifer and lactating cow

Particulars	Parameters	Heifer	Cow
Urine	pH**	7.70^B±0.12	8.63^A±0.13
	Colour	yellowish brown	Dark yellow
Dung	pH**	7.24^B±0.17	8.19^A±0.18
	Dm %	19.51±0.57	18.49±0.58
	Moisture %**	80.49^B±0.20	81.51^A±0.27

** Means with different superscripts in a row differ significantly (P<0.01)

Table 2: Microbial property of fresh urine and dung of heifer and lactating cow

Particulars	Parameters	Heifer	Cow
Urine	Total Bacteria 10^8 (cfu/ml)**	8.83^B±0.46	12.00^A±0.81
	Total yeast and mould 10^3 (cfu/ml)*	3.59^B±0.4	5.12^A±0.49
Dung	Total Bacteria 10^8 (cfu/g)*	16.17^B±0.7	20.16^A±1.58
	Total yeast and mould 10^3 (cfu/g)**	5.4^B±0.43	7.73^A±0.47

** Means with different superscripts in a row differ significantly (P<0.01)

* Means with different superscripts in a row differ significantly (P<0.05)

Conclusion

Among lactating cows and heifers, most of the physical parameters of fresh dung and urine did not differ significantly except for pH (8.63 ± 0.13). The total bacterial count (20.16 ± 1.58 and 12.00 ± 0.81) and yeast and mould count (7.73 ± 0.47 and 5.12 ± 0.49) were found to be significantly higher in cows dung and urine as compared to heifers with total bacterial count (16.17±0.7 and 8.83±0.46) and total yeast and mould count (5.40±0.43 and 3.59±0.40).

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