FACTORS ASSOCIATED WITH CHANGE IN PH, AMMONIA AND TOTAL NITROGEN OF MANURE MASS IN HIGH PERFORMANCE DAIRY COWS

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Abstract. The purpose of the present study was to monitor the changes in pH, ammonia and total nitrogen contents of manure samples obtained from three populations of dairy cows reared and fed in intensive production systems. The experiments were performed in April–May 2012 with three dairy cows' populations – A, B and C, fed 16, 17 and 18 % dietary crude protein and with 305-day milk yields of 25, 26 and 28 kg, respectively. It was established that the faecal pH of all three populations was between 6.07 and 6.65. The urinal samples showed alkaline values (pH urine 8.47 - 8.62). When urine was mixed with faeces, the ammonia nitrogen content increased correlating with manure mass pH increase. The average manure pH in the three studied populations attained 8.62 (population A), 8.48 (population B) and 8.49 (population C). Field analyses showed increase in manure pH from the beginning towards the end of the manure alley during cleaning. For population A, the respective values were 8.44 in the beginning, 8.88 in the middle and 9.05 at the end of the manure alley. After the passage of the scraper, the pH of manure remaining on the floor was 8.75. For the second population of cows, manure pH in the three manure alley points was 8.30; 8.42 and 8.64, and pH of remaining manure was alkaline (pH=8.50). For the third population, manure pH during scraping was 8.22; 8.52 and 8.78 in the beginning, middle and end of the manure alley respectively, while that of remaining manure on the floor was 8.54. Data showed that dairy cows were in an alkaline environment due to degradation of non-utilised urea nitrogen to ammonia nitrogen.

Abbreviations: MM, manure mass; CP, crude protein; TN, total nitrogen; NH_4-N, ammonia nitrogen; CO_2, carbon dioxide; NH_3, ammonia; NH_4OH, ammonium hydroxide

Keywords: pH, NH_4-N, N, CP, manure mass (MM); dairy cows, total nitrogen

Introduction. In a narrow sense, the term manure mass included faeces and urine excreted by animals. To increase milk yields, dairy cows are fed rations with high energy and protein content. The composition of rations is one of the factors which determine the content of nitrogen, phosphorus and potassium in faeces and urine.

In general, the utilisation of dietary nitrogen by ruminants is relatively non-efficient. In dairy cows, the nitrogen losses with urine decrease parallel to dietary crude protein content. This reduction has a minimum or insignificant effect on milk yields or milk protein content. The average efficiency of nitrogen utilization for milk is from 25 to 27 % (Bequette et al., 2003; Huhtanen and Hristov, 2009) and most of unutilized nitrogen is excreted with urine and faeces.

Olmos Colmenero and Broderick (2006) and Cyriac et al. (2008) established nitrogen utilization efficiency over 35 % in cows fed diets with crude protein level of only 13.5 % or 13.6 %. At the same time, the milk yield from cows fed rations with dietary CP from 15.0 to 18.5 % was not significantly influenced by CP level, whereas nitrogen output with faeces and urine increased proportionally to dietary CP (Groff and Wu, 2005).

The dietary crude protein level is essential for nitrogen output (in faeces and urine) (Frank and Swenson, 2002; Ishler, 2004; Hristov et al., 2011). High CP content contributes to excretion of considerable amounts of TN with urine and faeces (65–75 %), and at a lesser extent with milk (25–35 %) (Ishler, 2004). According to Todorov (2009), cows excrete half of the unused TN with urine, and 60–80 % of it is under the form of urea. Other studies (Reynal and Broderick 2005; Vander Pol et al., 2008) affirm that urea TN could attain 90% and even 92 % of total urinary nitrogen (Ganong, 1999).

When urine is mixed with faeces on the floor of barns, degradation of urea to CO_2 and NH_3 occurs under the influence of urease-producing faecal bacterial microflora (Ishler, 2004). Carbon dioxide is volatile and hence is evaporated, while ammonia remains in the manure mass (Sommer et al., 2006; Montes et al., 2009). Due to its high solubility in water, NH_3 reacts to form NH_4OH that alters manure pH (Hristov et al., 2011).

The intensive dairy cattle production systems are aimed at higher milk yields. This is inevitably related to consumption of more feed with high nutritive value. Nennich et al. (2005) proposed a formula for determination of the amount of MM on the basis of milk yield. According to Weiss (2004) the most important determinant of manure amount is the dry matter intake (DMI) of animals. Milk yield and dietary DMI are associated at a considerable extent, as high productivity is achieved with adequate DMI level.

The purpose of the present investigation was to monitor the changes of manure pH, as well as manure total and ammonia nitrogen in three population dairy cows in intensive production systems.

Material and methods

Equipment

The analysis of TN in manure samples was done with Kjeldahl digestion unit (VELP Scientifica DK 8S). NH_4-N was determined on automatic distillation system VELP SCIENTIFICA UDK 142. Laboratory pH measurements were performed with pH-meter Lab 850. Field determinations of manure pH in cattle farms were done with a portable pH-meter Universal Meter Multiline P_4.

Reagents and chemicals

The chemical analyses were conducted with analytical reagent-grade Merck and Fluka chemicals (0.1 N HCl; 20 % H_3BO_3; H_2SO_4 (d=1.84); 50 % NaOH; mixed indicator – 0.2 % alcohol solution of methylene blue and 0.25 % alcohol solution of methyl red; Cu catalyst tablets).

All glassware in contact with samples was soaked in HNO_3 solutions (1:1), left for at least 24 hours, and washed repeatedly with bidistilled water.

Analytical methods

pH determination: pH of samples was determined with a glass electrode in suspension as per Bulgarian State Standard ISO 10390 on a Lab 850 pH-meter.

Total nitrogen determination: Total nitrogen content of samples was done according to the classic Kjeldahl method which has undergone modifications with regard to temperature and time of digestion, acid concentrations and oxidation catalysts. The principle of the method consists in digestion of organic matter and reduction of nitrates with phenol-sulfuric or salicyl-sulfuric acid in the presence of catalysts, converting the nitrogen in samples to ammonium sulfate. The latter is digested by making the solution alkaline with addition of concentrated NaOH solution, and the liberated ammonia is distilled, reacts with boric acid of known concentration, and the excess is titrated with 0.2 N HCl. The analytical method has been modified and proposed from the manufacturer VELP Scientifics. The amount of samples, according to their TN content varied from 0.25 g to 1 g.

Analysis procedure: the respective amount of sample is weighed. To it, 7 g K_2SO_4, 5 mg Se, 7 ml H_2SO_4 and 5 ml H_2O_2 (hydrogen peroxide) are added. The thus prepared samples are placed in the digestion unit, previously set for 20-min digestion at 420 ^{0}C. After the digestion, the sample is distilled after being alkalised with 35% NaOH. The distillate is collected into a flask with 4% boric acid, the ammonia excess is titrated with 2N HCl, containing Toshiro's indicator. The used amounts of hydrochloric acid are used for calculation of total nitrogen content.

NH_4-N determination: Ammonia nitrogen was assayed on an automatic distiller VELP SCIENTIFICA UDK 142. All analyses were run in triplicate, at 20 ^{o}C.

Experimental animals:

In this experiment, three populations of dairy cows (A, B and C) reared in intensive production systems and fed a high-protein total mix ration were selected. Crude protein of rations was 16, 17 and 18 % for populations' A, B and C, respectively. The average 305-day milk yield of cows was 25, 26 and 28 kg. In each population there were at least 30 to 50 cows.

Samples:

Manure mass analyses were performed in April–May 2012. From each population, 6 urine and 6 faecal samples were collected from healthy animals within the same technological group. Samples were individually obtained using a device with a long handle and a container for collection of excreta during spontaneous urination and defecation. Thus, collected urine and faeces were not in contact either between them or with the barn floor. The three populations A, B, C were housed in free stalls, and manure was cleaned using a scraper device.

From individual containers, samples were transferred into clean tightly capper glassware and transported to the laboratory in a cool bag. One hundred ml of all urine samples within a population were taken and mixed to obtain a bulk sample. From faecal samples, 100 g were used and mixed in a similar manner. pH values of bulk samples were determined. Then a mixture of 2 parts faeces and 1 part urine (v:v) was prepared according to the proportion under natural conditions (Todorov, 2009), termed manure mass. At 3-hour intervals, one-third of the initial MM volume was left and was supplemented with two-thirds freshly prepared MM with a temperature of 38°C. This imitated most closely the conditions in cattle farms with regard to the continuing excretion of new amounts of faeces and urine by lactating cows. The pH values of these MM samples were determined at 60-min intervals over 24 hours. The urine and faecal samples were measured identically. Total nitrogen content of all MM samples from studied farms was assayed. The manure NH_4-N determination was done in the beginning, 3 hours later, immediately after adding fresh MM and so on for three experimental cycles with MM addition.

Field pH measurements were performed in the manure mass "wave" during alley cleaning (population A, B and C). Manure pH at farms was measured at the background of the identical hygienic parameters: air temperature and humidity. The measurements were done repeatedly in the beginning, the middle and the end of the manure alley. After the passage of the scraper, pH of manure remaining of the floor was also determined. After every field pH measurement, the electrode was washed with bidistilled water and wiped with a clean filter paper.

Data were analysed by analysis of variance (ANOVA) using the STATISTICA 6 software.

Results

The measurements of urine and faecal pH showed that for all three studied populations faecal pH was lower than 7, and urine pH values – higher than 7 (Table 1).

The analysis of results showed that at the time of mixing faeces with urine (2:1 ratio), the pH of resulting manure mass was close to the neutral value: population A – pH=7.56; population B – pH=7.62 and population C – pH=7.72. Until the 3rd hour, pH values attained 8.62 in population A; 8.58 in population B and 8.61 in population C. The average pH of manure from population A, B and C are presented on Fig. 1.

Table 1. **pH values of fresh** (at the time of excretion) **faeces and urine in studied populations**

Population	Number	Faecal pH		Urine pH	
		x ± SE	SD	x ± SE	SD
A	24	6.07 ± 0.011 [a]	0.054	8.47 ± 0.008 [a]	0.042
B	24	6.65 ± 0.023 [b]	0.112	8.62 ± 0.025 [b]	0.124
C	24	6.59 ± 0.011 [b]	0.057	8.54 ± 0.010 [bc]	0.050
Note: different superscripts indicate statistically significant differences – ab at P < 0.001; abc at P < 0.01					

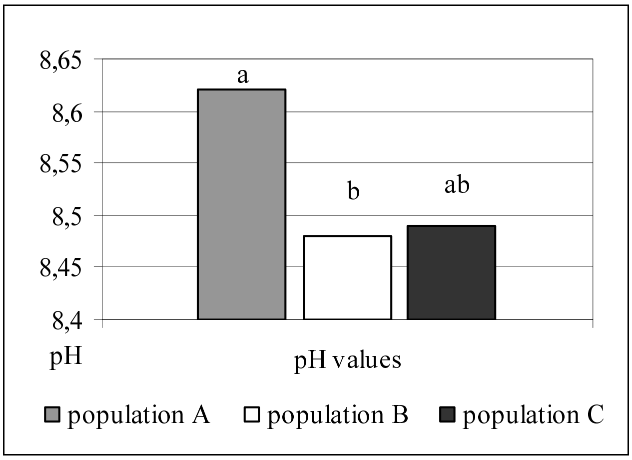

Fig. 1. **Mean pH values of manure mass from population A, B and C. Different superscripts indicate statistically significant differences – ab at P < 0.001**

The active reaction (pH) of tested MM samples varied from 8.48 (population B) to 8.62 (population A). In this experiment, the NH_4-N and N contents of manure were determined in the beginning, as well as on hours 3, 6 and 9. The results showed that until the 3rd hour, manure mass NH_4-N and N concentrations increased (Tables 2 and 3).

Immediately after addition of 2/3 parts of freshly prepared manure mass to 1/3 of the old one, NH_4-N and N values decreased, which resulted in lower pH of manure for the three populations. After a transient reduction of manure mass pH, the addition of fresh MM rapidly increased pH values (within 30 min) and attained values within the range 8.2 (population C) to 8.9 (population A).

The performed field studies showed variations in manure mass pH at different parts of the manure alley. Manure pH in the beginning of the alley were lower than those at its end for all three populations (P<0.01; Table 4).

Table 2. **NH_4-N content** (g/l) **of manure mass from studied populations**

Population	Number (n)	Hour 0		Hour 3		Hour 6		Hour 9	
		x ± SE	SD	x ± SE	SD	x ± SE	SD	x ± SE	SD
A	4	2.320± 0.44[Aa]	0.75	2.870± 0.05[Ab]	0.01	4.120± 0.05[Ac]	0.01	4.320± 0.05[Ad]	0.01
B	4	0.973± 0.06[Ba]	0.12	2.703± 0.03[Bb]	0.06	3.506± 0.01[Bc]	0.02	3.813± 0.01[Bd]	0.02
C	4	1.873± 0.02[Ca]	0.03	2.760± 0.06[Cb]	0.01	3.690± 0.05[Cc]	0.01	4.393± 0.03[Cd]	0.05
Note: different superscripts indicate statistically significant differences – ABC within a columns and abcd within a row at P < 0.05									

Table 3. **Total N content** (g/l) **in manure mass from studied populations**

Population	Number (n)	Hour 0		Hour 3		Hour 6		Hour 9	
		x ± SE	SD	x ± SE	SD	x ± SE	SD	x ± SE	SD
A	4	5.01± 0.005[Aa]	0.007	5.38± 0.001[Ab]	0.001	5.64± 0.01[Ac]	0.010	6.24± 0.010[Ad]	0.010
B	4	5.57± 0.010[Ba]	0.010	5.57± 0.014[Ba]	0.010	5.94± 0.02[Bc]	0.014	6.42± 0.012[Bd]	0.014
C	4	6.21± 0.012[Ca]	0.015	6.48± 0.01[Cb]	0.014	6.72± 0.02[Cc]	0.016	6.95± 0.011[Cd]	0.014
Note: different superscripts indicate statistically significant differences – ABC within a columns and abcd within a row at P < 0.05									

Table 4. **Manure mass pH in different parts of the manure alley for the three studied populations**

Populations	Number	Manure pH in the beginning of the alley	Manure pH in the middle of the alley	Manure pH in the end of the alley	pH of manure remaining on the alley
A	3	8.44 ± 0.0581^{Aa}	8.88 ± 0.0577^{Ba}	9.05 ± 0.0573^{Ca}	8.75 ± 0.0574^{Da}
B	3	8.30 ± 0.0576^{Ab}	8.42 ± 0.0582^{Bb}	8.64 ± 0.0580^{Bc}	8.50 ± 0.0571^{Cc}
C	3	8.22 ± 0.0567^{Ac}	8.52 ± 0.0578^{Bb}	8.78 ± 0.0578^{Cc}	8.54 ± 0.0565^{Dc}
Note: different superscripts within a column indicate statistically significant differences –a, b at P<0.01; a, c at P < 0.05. Significant differences at P < 0.05 within a row is marked with capital superscripts (A, B, C, D)					

Discussion

When faeces were mixed with urine on cattle barn floors, faecal microorganisms, through the enzyme urease, degraded urinary urea to ammonia and carbon dioxide (Bussink and Oenema, 1998; Ishler, 2004).

$$NH_2(CO)NH_2 + 3H_2O \rightarrow 2NH_4^+ + 2OH^- + CO_2 \rightarrow 2NH_3 + 2H_2O + CO_2$$

Factors influencing the rate of the reaction are the urea concentrations, ammonia cation concentrations, ambient temperature, environmental pH and the emitting surface (Muck, 1982; Van Vuuren and Jongbloed, 1994). While CO_2 is in the manure mass, the reaction is equilibrated but after carbon dioxide volatilisation, the equilibrium shifts to the right increasing the concentrations of NH_3 molecules interacting with water to obtain NH_4OH, which increases the alkaline character of MM.

Our data showed in Fig. 1 confirmed those of Burgos et al. (2010) reporting that pH of manure of cows fed rations with CP content between 17 and 19 % (the same protein level as in our experiments) varied from 8.49 to 8.69. The authors established that the amount of urine excreted by cows increased proportionally to dietary CP. Apart of the changes in the amount of urine, high-protein rations stimulate cows to drink more water (Church, 1979). The higher quantity of urine on the floor of animal barns is a prerequisite for more intensive chemical reactions in MM related to urea degradation, increase in NH_4-N concentrations and pH changes. According to our studies, manure mass pH when the ratio between urine and faeces in high-producing cows was 1:1 could attain 9.4 within 3 hours only (Penev et al., 2012). Muck (1982) provided evidence that the activity of urease depended very strongly on the ambient temperature. He showed that at a temperature of 30°C and urine to faeces ratio of 1:2.2 urea was degraded up to the 6th hour and pH attained 8.37 at hour 3.5. In modern feeding systems, in which urine output is equal to faecal one, a faster increase in pH could be anticipated. This was confirmed by the experiment with urine to faeces ratio of 1:2, resulting in pH values by the 3rd hours of 8.62; 8.58 and 8.61 in population A, B and C. During the warm months, when the air temperature indoor the barn often exceeds 30°C, the changes in manure pH would be particularly intensive attaining highly alkaline values. This is a precondition for extremely negative impact on claw horn properties through extraction of major part of horn fat followed by penetration of water, swelling and softening (Penev, 2013; Penev et al., 2014).

This was supported by other researchers, demonstrating lower hardness of claw horn under the influence of ammonium salts and alkaline pH of manure (Gregory et al., 2006; Higuchi et al., 2009). Milosavljević and Savić-Stefanović (2013) established a 74.01 % prevalence of hoof and claw diseases in cows housed in farms with very dirty and moist floors.

The laboratory analysis confirmed the results of Lee et al. (2009) who reported very low ammonia concentrations in fresh MM, but increased levels due to the rapid hydrolysis of urea (Table 2).

This correlated with periodical aggressive influence of pH on claw horn of dairy cows.

During the passage of the scraper, the mixing resulted in better contact between faeces and urine, hence urea degradation was enhanced and a rapid increase in manure pH occurred (Table 4) (Montes et al., 2009). This implies the use of adequate working schedule of scraper systems at cattle farms for maximum cleaning of manure mass. That is why pH of the manure mass pushed by the scraper towards the end of the alley was by 7 % higher for population A ($P < 0.01$), by 4 % for population B ($P<0.05$) and by 6.8 % ($P < 0.05$) for population C than values measured at the beginning (Table 4).

The use of slatted floors is a better technological solution for this problem, as urine and faeces could not accumulate on the farm floor and environmental pH would be maintained around the neutral one. The temperature of the manure mass in the duct should be kept within 16–18°C at a distance at least 15 cm from the slats with regard to release of less ammonia and carbon dioxide (Petkov et al., 1975). According to authors, slatted floors in cattle farms are a good solution as the conditions for manure mass in the duct reduce air ammonia concentrations 1.5 to 2 times.

Conclusion

The present study demonstrated that within 3 hours, manure mass underwent a chemical reaction causing increase in NH_4-N and TN concentrations, corresponding to alkalisation of the environment. The removal of 2/3 of the old manure mass and its replacement with fresh one resulted in reduction of NH_4-N and N, and subsequently, to lower manure pH for all three populations. The chemical reactions occurring in the slurry within 30 min after the addition of the fresh MM caused a novel increase in pH and NH_4-N. In farm ecosystems' environment, statistically significant differences were established in slurry pH the beginning and the end of the manure alley. The cleaning of MM with the scraper resulted in mixing and a better contact between faeces and urine. Consequently, the urea degradation was enhanced and manure pH at the end of the manure alley increased.

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