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Concentrations of Ammonia, Urea and Nitrate Nitrogen in Urine of Dairy Cattle in the University Herd

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Introduction

Currently, the adverse effects of nutrient losses from the manure of large dairy herds are of major concern to the quality of water and ground. Although they may be called wastes, these components are resources to be recycled. However, if these resources are in excess of the land application capacity, they are wastes. From an environmental standpoint, nitrogen is the nutrient of primary concern[5,6,7)].

In lactating dairy cows, 25 to 35 % of the nitrogen they consume is secreted into milk, and almost all the remaining nitrogen is excreted in feces and urine[6)]. Fecal nitrogen results from undigested feed, microbial protein and endogenous nitrogen. Urea and ammonia are the major forms of nitrogen excreted in urine. Urinary nitrogen is 40–50 % of the total nitrogen excreted from dairy cows[1,5,6)].

On the other hand, an excess of nitrogen applied to the land results in higher accumulation of nitrate in feed crops[3)]. Nitrate in the ration is usually reduced to ammonia, and is used by microbes in the rumen. However, if the nitrate content is very high, a considerable amount of nitrite is formed in the reduction process in the rumen, causing methemoglobin to form. Methemoglobin does not transport oxygen to the tissues[3)], and acute poisoning can kill the animal. There may be chronic poisoning causing abortion, poor conception rate and lameness in dairy cattle[3)].

The objectives of the current study were to determine the concentrations of urea and ammonia nitrogen in the urine from dairy cattle, and also the nitrate concentration in the urine in an apparently healthy dairy herd. The relationship between nitrate concentration and occurrence of several disorders in the herd are also discussed.

Materials and Methods

At the Research Farm of Rakuno Gakuen University, 79 dairy cows and heifers (73 Holstein and 6 Jersey) were used, including 63 milking cows, 4 dry cows and 12 heifers. The average milk yield of lactating cows at sampling day was 28.3±8.63 kg ranging from 7.0 to 42.7 kg. Days in milk ranged from 7 to 416 days. The cows were fed 4–5 kg DM from timothy silage, 3.5 kg DM from corn silage and 5.6 kg DM from alfalfa silage. In addition, the lactating cows were fed 1.8 kg DM from beet pulp and a certain amount of concentrate mixture to fulfill energy requirements[1)]. The average feeding level of crude protein for lactating cows was 3.15 kg daily, and about 120% of the requirement[1)].

Urine samples were collected during urination from each cow before morning feeding at 5:30 because it was expected that urinary nitrate was hardly detectable in cows with normal ruminal reduction ability. Urinary pH was determined with a glass electrode pH meter immediately after sampling. Samples were kept at 5 ℃ for analysis within 6 hours after sampling, or at −20 ℃ for later analysis. Urinary ammonia nitrogen was measured by steam distillation with a Kjeldahl apparatus, and ammonia plus nitrate

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nitrogen was measured with Devarda's metal with the same apparatus. The difference between these measurements was regarded as nitrate nitrogen. In the present study, ammonia nitrogen meant ammonia plus ammonium nitrogen. Urinary urea nitrogen was measured with a clinical kit (Urea Nitrogen-Test Wako; Wako Pure Chemical Industries) after 20-fold dilution for 43 samples.

After extraction with distilled water, nitrate and nitrite concentrations in feeds were measured by high-pressure liquid chromatography (HPLC). Nitrate and nitrite in the drinking water were checked with a pack-test kit for water inspection (Kyoritsu Chemical-check Lab.).

Occurrence of disorders and average days open were analyzed by chi-square test and Student's t-test, respectively.

Results and Discussion

The pH of the urine samples ranged from 8.14 to 8.63 and averaged 8.41 ± 0.095. These values were somewhat higher than the normal pH of bovine urine, which is generally considered to be 7.4–8.4[2]. The pH of urine may increase because of formation of ammonia from urea. However, in this study the pH was measured immediately after collection of urine. Bovine urine was also reported to have a pH value between 8.0 and 8.5 by Nakamura[4]. The most of the present results were in this range.

The major component of nitrogenous compounds in urine is urea[4]. The average concentration of urea nitrogen was 5465 ± 1606 mgL^{-1} with the range from 3148 to 10835 mgL^{-1}. The histogram is shown in Fig. 1. The mode was between 5000 and 5500 mgL^{-1}. Usually, about 85–90 % of total nitrogen in urea is urea nitrogen[4]. The typical Holstein cow consuming 17.8 kg DM and producing 22.7 kg milk has been reported to excrete 22.7 kg urine with a total nitrogen concentration of 6 gkg^{-1} [6]. This value is equivalent to 5100–5400 mgL^{-1} urea nitrogen. More recent reports indicate that dairy cows excrete 13.0–27.2 kg urine containing 5.65–9.37 gkg^{-1} total nitrogen[1,7]. There are considerable variation in the amount of urinary excretion of nitrogen, depending on many factors such as crude protein intake, milk production, water consumption and ambient temperature.

In the present study, ammonia nitrogen concentration was 140.8 ± 38.0 mgkg^{-1} with the range from 71 to 298 mgkg^{-1}. Fig. 2 illustrates the distribution of ammonia nitrogen concentration in the urine of the cows. Given that about 20–40 gkg^{-1} of total nitrogen in urine was ammonia nitrogen[4], the total nitrogen concentration (6.0 gkg^{-1}[1]) would indicate that the ammonia nitrogen level is expected to be 120–240 mgkg^{-1}. It was considered that the present data were reasonable. There was close positive relationship between the concentrations of urea nitrogen and ammonia nitrogen (Table 1).

In 24 cows out of 79, nitrate nitrogen was detected in urine (Fig. 3). The concentration was 11.5 ± 8.6 and between 1 and 37 mgkg^{-1}. The coefficient of variation was 0.74. There were no significant correlations between nitrate nitrogen

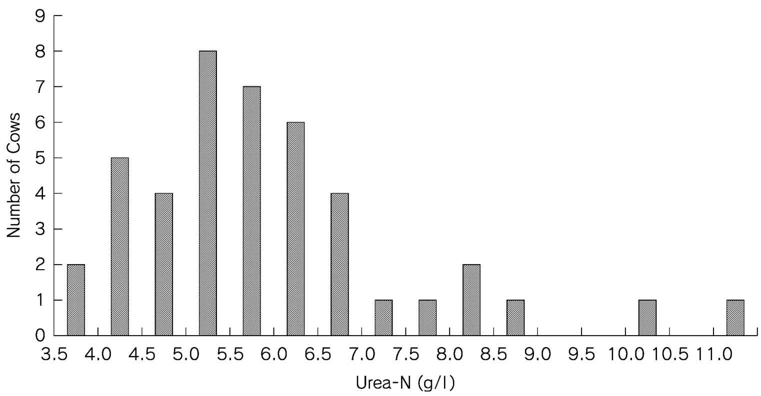

Fig. 1 Distribution of urea-nitrogen concentration in urine of dairy heifers and cows

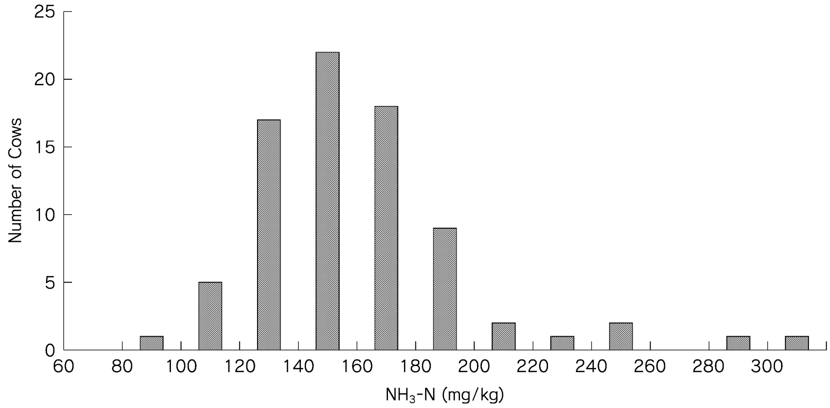

Fig. 2 Distribution of ammonia nitrogen concentration in urine of dairy heifers and cows

and urine pH, urea nitrogen and ammonia nitrogen (Table 1). The cows consumed rations containing less than 400 $mgkg^{-1}$ nitrate nitrogen in dry matter, and consumed less than 8 g nitrate nitrogen per day (4.78 ± 2.09 gd^{-1})[1]. The nitrate content of corn silage was 210–559 $mgkg^{-1}$, and grass silage contained 31–260 $mgkg^{-1}$ in dry matter. Concentrate contained less than 100 $mgkg^{-1}$ except beet pulp, which contained 219 $mgkg^{-1}$. It is believed that rations containing less than 5000 $mgkg^{-1}$ or at least 1000 $mgkg^{-1}$ is safe[3]. Drinking water did not contain nitrate. The feeding level of nitrate was considered to be normal and safe.

Table 1 Correlation coefficient among urine pH, urea, ammonia and nitrate nitrogen in urine of dairy cows and heifers

	Urea-N	NH_3-N	NO_3-N
Urine pH	0.117	0.133	0.017
Urea-N		0.738**	−0.229
NH_3-N			−0.192

＊＊：$p < 0.01$

The correlation coefficient between nitrate consumption and urinary nitrate concentration was only 0.135. In the present study, urine samples were taken 13 hours after the last feeding. It was considered that healthy cows with higher reduction ability of nitrate in the rumen excreted urine without nitrate at that time. Cows excreting urine containing nitrate might have lower reduction ability in the rumen, thus forming nitrite in the reduction process, which would cause methemoglobin to form[3]. In such case, nitrate might be excreted in urine for a longer period.

In the various disorders encountered in the last year, no significant difference was noted between the cows excreting urine with nitrate and those excreting urine without nitrate (Table 2). Reproductive disorders in the cows without nitrate tended to be more prevalent than in the others. More than half the disorders involved stillbirth.

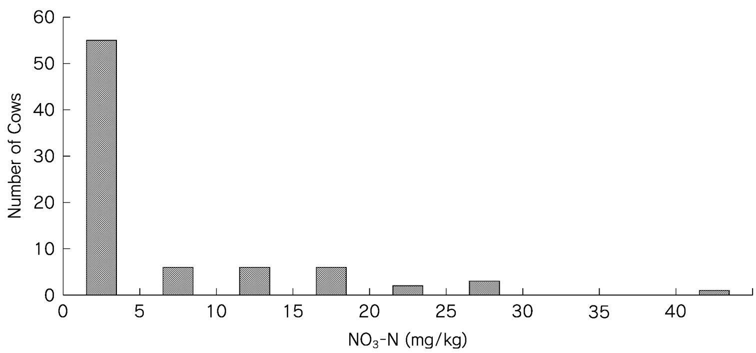

Fig. 3 Distribution of nitrate nitrogen concentration in urine of dairy heifers and cows

Table 2 Disorders recorded in dairy cows with and without nitrate ditected in the urine

	$+NO_3$-N (%; n=24)	$-NO_3$-N (%; n=55)	Significance
Reproductive disorders	8.3	21.8*	NS
Metabolic disorders	20.8	20.0	NS
Mastitis	12.5	7.3	NS
Lameness	4.2	9.1	NS
Total disorders	54.2	58.2	NS
Days from calving to conception	111.8	93.7	$p<0.05$

*: Stillbirth was more than half of the disorders.

The cause of the stillbirth was not clear. However, days open was greater in the cows in which nitrate was detected than in the other cows (111.8 ±37.6 days vs. 93.7 ±35.0 days; $p < 0.05$). There might be some negative effects of methemoglobin formation on conception or on continuance of early pregnancy[3].

In the present study, only the concentrations of urea nitrogen, ammonia nitrogen and nitrate nitrogen in urine were measured, and no information on the amount of excretion of such nitrogenous compounds. Urine volume is affected by many factors; consequently the concentrations of nitrogenous compounds may vary considerably. Because of shortage of information, however, the data on the relative participation of nitrogenous compounds in urine might be helpful to solve environmental problems in dairy herds, and the data on nitrate in the apparently healthy herds might be helpful to maintain cow's health.

Summary

Urine samples were collected from 79 dairy cows (63 lactating, 4 dry and 12 heifers) at 13 hours after the last feeding to determine the pH value and concentrations of urea nitrogen, ammonia nitrogen and nitrate nitrogen. The pH ranged from 8.14 to 8.63, and the average was 8.41± 0.095. Average concentrations of urea and ammonia nitrogen were 5465±1606 mgL^{-1} and 140.8±38.0 $mgkg^{-1}$, respectively. In 24 of the cows, nitrate nitrogen was detected, and the concentration was 11.5±8.6 $mgkg^{-1}$. A close positive correlation was found between the concentration of urea nitrogen and that of ammonia nitrogen. Nitrate content of dry matter in the ration was less than 400 $mgkg^{-1}$, and nitrate consumption by the cows was less than 8 g per day. This was considered to be safe level. The correlation coefficient between nitrate nitrogen consumption and the urinary concentration of nitrate was 0.135. In the frequency of disorders within the last year, no difference was observed between the cows in which nitrate was found in the urine and the other cows. However, the days open were considerably greater in the cows with nitrate detected in the urine than in the other cows (111.8 vs. 93.7; $p < 0.05$).

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要　約

酪農学園大学附属農場の乳牛群(搾乳牛 63 頭，乾乳牛 4 頭，育成牛 12 頭)の尿を最後の飼料給与 13 時

間後の午前5時30分に採取し，pH値，尿素窒素，アンモニア態窒素および硝酸態窒素濃度を測定した。尿のpHは8.41±0.095であり，範囲は8.14～8.63であった。尿素態窒素濃度は5,465±1,606 mgL^{-1}であり，範囲は3,148～10,835 mgL^{-1}であった。アンモニア態窒素濃度は140.8±38.0 $mgkg^{-1}$であり，範囲は71～298 $mgkg^{-1}$であった。79頭中，24頭の尿から硝酸態窒素が検出された。検出された尿の硝酸態窒素濃度は11.5±8.6 $mgkg^{-1}$であり，範囲は1～37 $mgkg^{-1}$であった。尿の尿素態窒素濃度とアンモニア態窒素濃度との間には高い正の相関が認められた。他の窒素成分の間には有意な相関は認められなかった。飼料の硝酸態窒素濃度は乾物中400 $mgkg^{-1}$以下であり，1日の摂取量は8 g以下と安全とされる水準であった。硝酸態窒素の摂取量と尿の硝酸態窒素濃度の間の相関は低かった（r＝0.135）。尿中に硝酸態窒素が検出された牛と検出されなかった牛の過去1年間の疾病率に差は認められなかった。しかし，硝酸態窒素検出牛の空胎日数（111.8日）は検出されなかった牛（93.7日）より有意に長かった（$p<0.05$）。