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Original Article

# Dietary nitrate loads on cows in dairy farms near Lake Dian, Kunming City, Yunnan Province, China.

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## Summary

In a cropland area near Lake Dian, large amount of the crop wastes, i.e. vegetable and flower residues, were fed to cows in dairy farms. Since several vegetables and flowers tend to accumulate nitrate, animals kept such situations were suspected to ingest large amount of nitrate. The objective of this study was to quantify the dietary nitrate loads on dairy cows in the area. Questionnaire surveys on feedstuffs fed to cows were conducted in the dairy farms from October 2009 to September 2010. Nitrate contents in the feedstuffs and methemoglobin concentrations in the blood of cows were measured. The feeding amounts of crop wastes from vegetables and flowers were 28.4 kg to 50.0 kg per head per day on a fresh weight basis. The contents of nitrate nitrogen in the residues of rape blossoms, parsley, stem lettuce and carnations were 0.19％DM to 0.37％DM, which were potentially toxic levels for ruminants. Those in the residues of celery and broccoli were 1.36％DM and 0.59％DM respectively, which were toxic levels. However, the nitrate nitrogen contents in the total diets (sum of feed ingredients) were 0.15％DM to 0.19％DM, which were below the allowable limit. The ratios of methemoglobin to the total hemoglobin in the blood were less than 10％, indicating that the dairy cows did not fall into acute nitrate poisoning.

## Introduction

There have been numerous reports of deaths in ruminant animals due to nitrate toxicity[1)]. Nitrates ($NO_3^-$) ingested by ruminant animals are generally reduced to ammonias through nitrites ($NO_2^-$) and hydroxylamines in the rumen[2)]. However, when dietary nitrate levels are especially high, nitrites are directly transferred from the rumen to the blood. The nitrites in the blood convert ferrous ions ($Fe^{2+}$) of hemoglobin to ferric ions ($Fe^{3+}$), and methemoglobin is produced[3)]. Due to the inability of methemoglobin to carry oxygen, animals begin to present symptoms of oxygen deficiency and cyanosis as the ratio of methemoglobin to total hemoglobin rises; at very high ratios, they will die[4)]. This is the process of nitrate poisoning in ruminants.

Kunming city, the capital of Yunnan province, China, has a population of 6 million people. In the southeastern shore areas of Lake Dian, located 40 kilometers from the center of Kunming city, vegetable and flower production has expanded to meet the increasing demand in the urban area. In this area, large amounts of chemical fertilizer and animal manure are applied to the cropland. Since several vegetable and flower species have a tendency to uptake and deposit nitrates from the soil[5)], it is believed that some harvests might have high levels of nitrate. The crop residues and the market irregulars from the vegetables and flowers are supplied to cows in dairy farms in the area, and therefore, the dairy cows were suspected of having ingested excessive quantities of nitrates and of being affected by nitrate poisoning. The objective of this study was to quantify the dietary nitrate loads on the dairy cows by surveying the amounts of feed ingredients, analyzing nitrate contents in the feedstuffs and determining methemoglobin levels in the blood of cows.

## Materials and Methods

### Site description

The investigation was conducted in the cropland area near the southeastern shore of Lake Dian (24.7°N, 102.7°E, 1892 m above sea level), Kunming city, Yunnan province,

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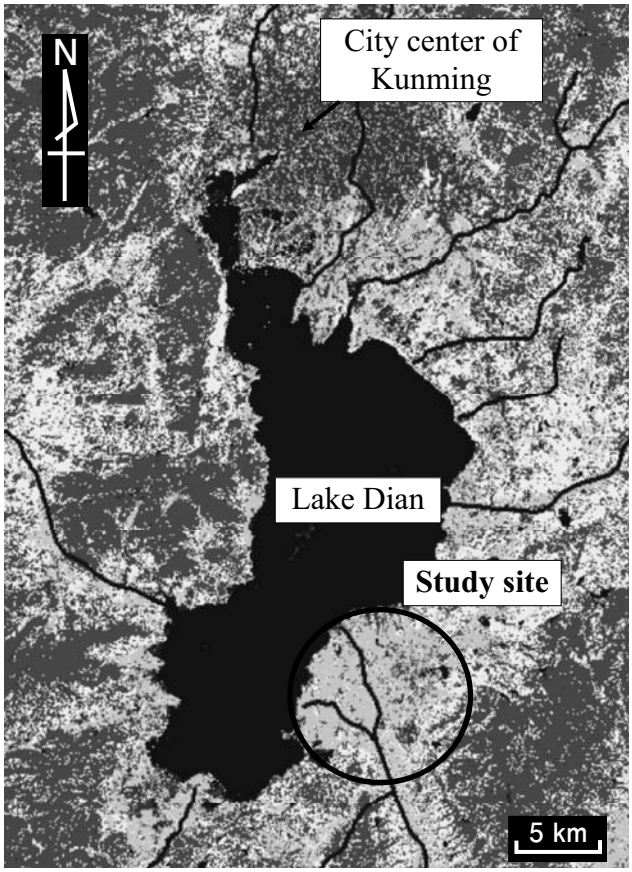


**Fig. 1** Location of the study site.

China (Fig. 1). Because of the high altitude, this area has been classified as temperate monsoon climate despite the low latitude, and the annual mean temperature and precipitation are 15.0 °C and 1,017 mm, respectively. The area consists of 27 administrative villages located 40 km from the city center of Kunming. The total population and the number of households in the area are 53,677 and 17,714, respectively.

Table 1 shows the agricultural characteristics of the study area, obtained from the investigation. Cultivation of vegetables and flowers is very popular in the area, and most of these crops are cultured in greenhouses. The area covered by greenhouses is about 85% of the total cultivated area.

**Table 1** Agricultual characteristics of study area

| Land area (ha) | | No. of animals (head) | |
|---|---|---|---|
| Grain | 167 | Dairy cattle | 4,034 |
| Vegetable | 1,664 | Pig | 36,564 |
| Flower | 895 | Chicken | 293,155 |
| Other | 73 | Duck | 58,219 |
| Total | 2,799 | | |

**Field survey and sample collection**

The amount of each feedstuff fed to cows at each of 5 dairy farms (Farm A to E) in the area was investigated by interview from October 2009 to September 2010 (Farm A and B: March, May and September 2010, Farm C: October 2009, Farm D: March 2010, Farm E: March and June 2010). Each feedstuff was sampled and subsequently dried at 70°C for 48 hours to measure the dry matter percentage. Blood samples of lactating cows from 2 farms (Farm A and B) were also sampled using heparinized vacuum tubes.

**Chemical analysis**

The feed samples were ground using a Wiley mill, added to distilled water, shaken at 45°C at 1 hour, and filtered. Nitrate nitrogen contents in the samples were analyzed by the Cataldo method[6]. Dietary nitrate nitrogen amount per head per day was estimated by multiplying the nitrate nitrogen content in each feedstuff by its amount fed and summing the products. At the calculation, all "vegetable wastes" were assumed to be broccoli wastes because the supplied vegetable residues differed from day to day, but broccoli wastes were available throughout the year.

Whole blood samples were hemolyzed by distilled water and added to M/10 phosphoric acid buffer, and the supernatant was separated by centrifugation at 3,000 rpm for 10 minutes. Ratios of methemoglobin to the total hemoglobin in the blood samples were determined by a colorimetric method using a visible-ultraviolet spectrophotometer with 5% sodium cyanide solution and 20% potassium ferri-cyanide solution based on the cyan-methemoglobin method[7].

## Results and discussion

The amounts of each feedstuff and the proportions of vegetable and flower wastes in the whole feed at 5 dairy farms are shown in Table 2. Fermented corn residue, maize powder, rice bran, noodle powder, yellow bean cake, concentrate mixture and wheat bran were fed to dairy cows as concentrates. Broad bean stems, rice straw and maize stems and leaf silage were fed as roughages. Vegetable and flower wastes, residues on cropland and market irregulars available at the moment, such as broccoli, celery, parsley, stem lettuce, purple cabbage, rape blossoms and carnations, were also fed to dairy cows. The fed amounts of these wastes were 28.4 kg to 50.0 kg per head per day in fresh weight, and their proportions out of the whole feed were 25% to 31% on a dry weight basis. Most of the dairy farmers in the area did not have their own cultivated lands for feed crops; thus, the vegetable and flower wastes obtained from nearby sources were useful in terms of price and availability compared with feed purchased far from their farms. As a result, most of the dairy farms in

**Table 2** Amounts of feedstuffs and proportions of vegetable and flower wastes at 5 investigated farms

| | Farm A | | Farm B | | Farm C | | Farm D | | Farm E | |
|---|---|---|---|---|---|---|---|---|---|---|
| Feeding amount (kg/day/head) | FW[1)] | DM[2)] | FW[1)] | DM[2)] | FW[1)] | DM[2)] | FW[1)] | DM[2)] | FW[1)] | DM[2)] |
| Concentrates | | | | | | | | | | |
| Fermented corn residue | 10.0 | 2.3 | 8.0 | 1.8 | 5.0 | 1.1 | | | | |
| Maize powder | 4.5 | 4.0 | 8.0 | 7.1 | 5.0 | 4.5 | 5.0 | 4.5 | 5.0 | 4.5 |
| Rice bran | 1.0 | 0.9 | 2.0 | 1.8 | | | | | | |
| Noodle powder | 0.5 | 0.4 | | | | | | | | |
| Yellow bean cake | 5.0 | 0.5 | | | | | | | | |
| Concentrate mixture | | | | | 3.0 | 2.7 | | | | |
| Wheat bran | | | | | | | 1.5 | 1.4 | | |
| Roughages | | | | | | | | | | |
| Broad bean stems | 1.0 | 0.8 | 2.0 | 1.7 | | | | | 2.0 | 1.8 |
| Rice straw | 1.1 | 1.1 | 3.0 | 2.9 | | | 1.5 | 1.4 | | |
| Maize stems and leaf silage | 8.4 | 1.9 | | | | | | | | |
| Vegetable and flower wastes | 43.0 | 5.0 | 50.0 | 5.8 | 30.0 | 3.5 | 28.4 | 3.3 | 28.7 | 3.3 |
| Total | 74.5 | 16.9 | 73.0 | 21.1 | 43.0 | 11.8 | 36.4 | 10.5 | 35.7 | 9.6 |
| Proportion of vegetable and flower wastes in total feed (%) | 57.7 | 29.5 | 68.5 | 27.5 | 69.8 | 29.4 | 78.0 | 31.4 | 80.4 | 34.5 |

[1)]Fresh weight, [2)]Dry matter weight

the area have used these wastes as feed. To our knowledge, there have been no previous reports about situations in which fresh vegetables and/or flowers comprised such a large part of dairy cows' diets.

The nitrate nitrogen content in each feed is shown in Table 3. The nitrate nitrogen contents in concentrates and roughages were less than 0.05%DM, which were within the range of the safety standard of nitrate nitrogen contents in feedstuffs for ruminants (< 0.1%DM)[8)] (Table 4). The nitrate nitrogen contents in parsley, stem lettuce, rape blossoms and carnations were 0.19%DM to 0.37%DM. These residues were at potentially toxic levels by the standard (0.15%DM to 0.4%DM)[8)] and therefore should be restricted in feeding amount, The nitrate nitrogen contents in celery and broccoli were 1.36%DM and 0.59%DM, respectively. These residues should not be fed to ruminants to prevent acute toxicosis (> 0.4%DM)[8)]. The nitrate nitrogen contents in the total diets in the 5 farms were 0.15%DM to 0.19%DM, which were below the allowable limit given by MAFF (< 0.2%DM)[9)]. It is known that ruminants often fall into nitrate poisoning when they ingest soiling crops containing high levels of nitrates in a short period[10)]. Therefore, it was suggested that occurrence of acute toxicosis for the dairy cows may depend on the feeding management, ingredients and intervals.

**Table 3** Nitrate nitrogen contents in feedstuffs (% DM)

| Items | n | Mean | SD[1)] |
|---|---|---|---|
| Concentrates | | | |
| Fermented corn residue | 4 | 0.002 | 0.0023 |
| Maize powder | 3 | 0.003 | 0.0035 |
| Oilseed cake | 1 | 0.002 | — |
| Roughages | | | |
| Broad bean stems | 2 | 0.000 | 0.0001 |
| Rice straw | 2 | N.D.[2)] | 0.0000 |
| Maize stems and leaf silage | 1 | 0.050 | — |
| Vegetable wastes | | | |
| Celery | 3 | 1.357 | 0.3615 |
| Broccoli | 5 | 0.592 | 0.2238 |
| Rape blossom | 1 | 0.368 | — |
| Parsley | 2 | 0.248 | 0.0880 |
| Stem lettuce | 1 | 0.190 | — |
| Purple cabbage | 1 | 0.138 | — |
| Tomato | 1 | N.D.[2)] | — |
| Flower wastes | | | |
| Carnation | 3 | 0.260 | 0.1005 |

[1)] Standard deviation.
[2)] Not detected.

The ratios of methemoglobin to the total hemoglobin were less than 10% (Table 5). In humans, the normal level of methemoglobin is 1 to 2% of the total hemoglobin; symptoms of cyanosis will appear at 10%, consciousness is lost at 50%, and death occurs at 70%[11)]. This also holds true in dairy cows[12)]. Therefore, in the present study, the dairy cows were assumed not to have fallen into acute nitrate poisoning.

**Table 5** Ratios of methemogrobin to the total hemoglobin in blood of cows (%)

| Sampling date | n | Methemoglobin proportion[1)] |
|---|---|---|
| Jun-2010 | 5 | 2.2 (1.6 – 3.2) |
| Sep-2010 | 3 | 5.0 (3.0 – 8.0) |

[1)] Mean (min. – max.).

**Table 4** Safety standard of nitrate nitrogen content (% DM) in feedstuffs for ruminants

| Nitrate nitrogen content | Effect on ruminants |
|---|---|
| < 0.10 | Safe. |
| 0.10 to 0.15 | Moderately safe. Limit use for pregnant animals to 50% of total ration. |
| 0.15 to 0.20 | Potentially toxic. Limit feed to 50% of total ration. |
| 0.20 to 0.35 | Potentially toxic. Limit feed to 35% of total ration. Do not feed to pregnant animals. |
| 0.35 to 0.40 | Potentially toxic. Limit feed to 25% of total ration. Do not feed to pregnant animals. |
| > 0.40 | Toxic. Do not feed to prevent death and acute toxicosis. |

Quated from Bradley *et al.* (1940)[8)]

Although the methemoglobin levels in the blood did not imply acute nitrate poisoning, some vegetable wastes had toxic levels of nitrates, and the nitrate contents in the whole diets were close to the allowable limit. The high methemogrobin levels in September, 2.3 times as high as that in June, might have reflected high nitrate contents in the diets at the moment. Even if it does not cause acute poisoning, frequent intake of relatively high levels of nitrate may cause chronic nitrate poisoning and thereby affect reproduction, lactation and growth: namely, increased rates of abortion and fetal abnormality, decrease of milk yield and body weight gain[13]. Therefore, the dairy farmers in the area should pay attention to the potential for nitrate poisoning in their dairy cows and give special consideration to the variety and proportions of vegetable and flower wastes in their diets.

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