

Evaluation of nutrient flows in animal production in
the southeastern basin of Dianchi Lake, Yunnan Province, China

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2016

Evaluation of nutrient flows in animal production in
the southeastern basin of Dianchi Lake, Yunnan Province, China

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A dissertation presented to the Office of Graduate School of Agriculture

Kyoto University

In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

January 2016

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of Dianchi Lake, Yunnan Province, China

Doctoral thesis

Hiroki Anzai, 2016

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ABSTRACT

During the last two decades, agricultural production systems have been greatly intensified in the southeastern basin of Dianchi Lake, one of the most eutrophied lakes in China. The agricultural intensification may result in nutrient discharge from the croplands, along with associated problems in agricultural production. The objective of this thesis was to evaluate nutrient flows in animal production in the southeastern basin of Dianchi Lake. Major nutrient budgets (nitrogen, phosphorus, potassium and magnesium) were calculated for dairy cattle, fattening pigs, breeding sows, broilers and laying hens based on interview surveys with farmers and chemical analyses for nutrient contents in sampled feeds. The obtained budgets were extended to describe nutrient flows through the entire animal production system. Although nutrients from local feed materials were utilized in the dairy and fattening pig production systems, nutrients in manure exceeded nutrients used as local feeds within the entire area. The findings indicated that animal production might contribute to imbalances in nutrient budgets on the croplands through manure application. Moreover, the results of the quantification of dietary nitrate load on dairy cattle suggested that there may be a risk of nitrate poisoning due to massive feeding of vegetable and flower residues produced in the area. Comprehensive approaches to appropriate nutrient management both in crop and animal production systems are required for sound and sustainable nutrient cycling in regional agricultural systems.

Key words: regional nutrient flow, nitrogen, phosphorus, potassium, magnesium, nitrate poisoning,

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LIST OF PUBLICATIONS

Chapters 2 and 3 are the peer reviewed version of the following articles, respectively:

Anzai H, Wang L, Oishi K, Irbis C, Li K, Kumagai H, Inamura T, Hirooka H. 2015.

Estimation of nitrogen and phosphorus flows in livestock production in Dianchi Lake basin, China. *Animal Science Journal*.

Amachika Y, Anzai H, Wang L, Oishi K, Irbis C, Li K, Kumagai H, Inamura T, Hirooka,

H. 2015. Estimation of potassium and magnesium flows in animal production in Dianchi Lake basin, China. *Animal Science Journal*.

, which have been published in final forms at <http://dx.doi.org/10.1111/asj.12390> and <http://dx.doi.org/10.1111/asj.12518>, respectively

These articles may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Chapter 4 is the peer reviewed version of the following article:

Anzai H, Oishi K, Li K, Irbis C, Hirooka H, Inamura T, Kumagai H. 2011. Dietary nitrate

loads on cows in dairy farms near Lake Dian, Kunming city, Yunnan Province, China. *Trace Nutrient Research* 28, 54-57.

, which has been published in final form at <http://www.jtnrs.com/sym28/P-07.pdf>.

ACKNOWLEDGEMENTS

The author wishes to acknowledge sincere gratitude to Professor Dr. Hiroyuki Hirooka of Kyoto University, for guiding me in the study and unreserved commitment to bring me to the completion of this thesis, and to Associate Professor Dr. Hajime Kumagai of Kyoto University and Assistant Professor Dr. Kazato Oishi for their advices and suggestions on the study. Without their guidance, this study could never have been completed.

Acknowledgements are extended to Professor Dr. Tatsuya Inamura of Kyoto University, for leading me in the research project in China, and to Professor Dr. Kunzhi Li and Professor Dr. Chagan Irbis of Kunming University of Science and Technology, for enabling me to conduct surveys in China. I also would like to express appreciation to Mr. Yuta Amachika, Miss Lin Wang and all the collaborators of the project for their cooperation in the surveys.

I am also grateful to former and current members of the Laboratory of Animal Science Resources for their encouragements and friendship. Finally, I am filled with gratitude to my family for their generous financial and mental supports.

Hiroki Anzai

January, 2016

CHAPTER 1

General introduction

1.1 Problems associated with intensive agricultural production

Over the past several decades, productivity in crop and animal production has been greatly improved by intensification through increasing nutrient inputs into agricultural systems as chemical fertilizers and concentrated feed. These productivity improvements have filled the increasing demands for agricultural and livestock products associated with population growth and economic development. However, the excess nutrient inputs have several negative aspects. Excessive nutrients that are not fully utilized in the production systems result in environmental loads at regional and global scales. Nitrogen (N) and phosphorus (P) outflowing from farmlands into aquatic systems cause water pollution and eutrophication (Van Horn *et al.* 1996). Nitrogen emitted to the atmosphere as ammonia during composting and spreading of manure is a factor in acidification, water pollution and eutrophication following re-deposition to soils (Mosquera *et al.* 2005). Carbon, as carbon dioxide and methane, and nitrogen, as nitrous oxide, emitted to the atmosphere from animal barns, manure storage sites and farmland soils are major contributors to global warming. In addition to the environmental concerns, excess inputs of some nutrients can prevent healthy growth of crops or animals and may also be poisonous to humans who use these products. For example, high potassium (K) content in soils and subsequently in feeds may lead to milk fever in dairy cattle and grass tetany in cattle (National Agriculture and Food Research Organization (NARO) 2008). As a result of high nitrate contamination in soils and agricultural products, excess ingestion of nitrate (NO_3^-) causes methemoglobinemia in animals and humans, which leads to cyanosis and possibly death due to the inability of methemoglobin to carry oxygen (Hungerford 1970). In the coming decades, agricultural intensification with accompanying problems will

continue to occur and expand primarily in developing countries in response to increasing food demands.

1.2 Lake water eutrophication in China

In China, lake water eutrophication has become one of the most important factors impeding sustainable economic development and affecting people living in the associated catchment area (Le *et al.* 2010). Water eutrophication can result in frequent outbreaks of algal blooms, and in the worst cases, it can threaten the drinking water supply. Most freshwater lakes in the central and lower reaches of the Yangtze River have been eutrophied through the accumulation of excessive nutrients such as N and P as a consequence of rapid economic development coupled with the over-exploitation of water resources since the 1980s (Qin 2002). In an investigation of 67 lakes around China, Li (2006) reported that 80% of the studied lakes had been polluted to a level unhealthy for human contact; only approximately 20% of the study lakes were of a relatively good quality. There are two primary modes of nutrient discharge into lakes from external sources, point discharge and nonpoint discharge. Nonpoint discharge, such as waste water from agriculture, aquaculture, rural dwellers, and soil erosion, is much more difficult to handle than point discharge, which includes industrial waste water and domestic sewage from fixed locations (Le *et al.* 2010). Approximately half of the pollutants discharged into many Chinese lakes originated from nonpoint sources (Jin 2001). Among the nonpoint discharge sources, agricultural waste water has an overwhelming effect (Gao *et al.* 2002). The annual amounts of chemical fertilizers applied in China in 2010 were 3.5×10^7 t N

and 1.7×10^7 t P_2O_5 ; these are the largest amounts of fertilizers applied in the world and account for approximately one-third of the total global use (FAO 2012). Thus, the environmental impacts by N and P losses from agricultural lands in China might be a serious problem on both regional and global scales.

Dianchi Lake, located to the south of Kunming, the capital of Yunnan Province, is the sixth largest lake in China and is an essential water source for thousands of local residents (Figure 1.1). Dianchi Lake is a depression lake with a surface area of 300 km², a catchment area of 3000 km², a maximum water depth of 10.9 m and an average water depth of 4.4 m (Tanaka *et al.* 2013). Due to the development in the catchment area, this lake is one of the three most eutrophied lakes in China, along with Lake Taihu and Chaohu (Liu & Qiu 2007). According to monitoring data from 2005 to 2012, concentrations of total N ranged from 1.82 to 3.01 mg/L, and total P ranged from 0.13 to 0.20 mg/L in the main water body of the lake (Zhang *et al.* 2013). Among the nutrients entering the lake, 45% of the N and 27% of the P were considered to have originated from agricultural fields (Wan *et al.* 2007). Therefore, control of nutrient discharge from agricultural fields is the key to bringing lake water eutrophication under control.

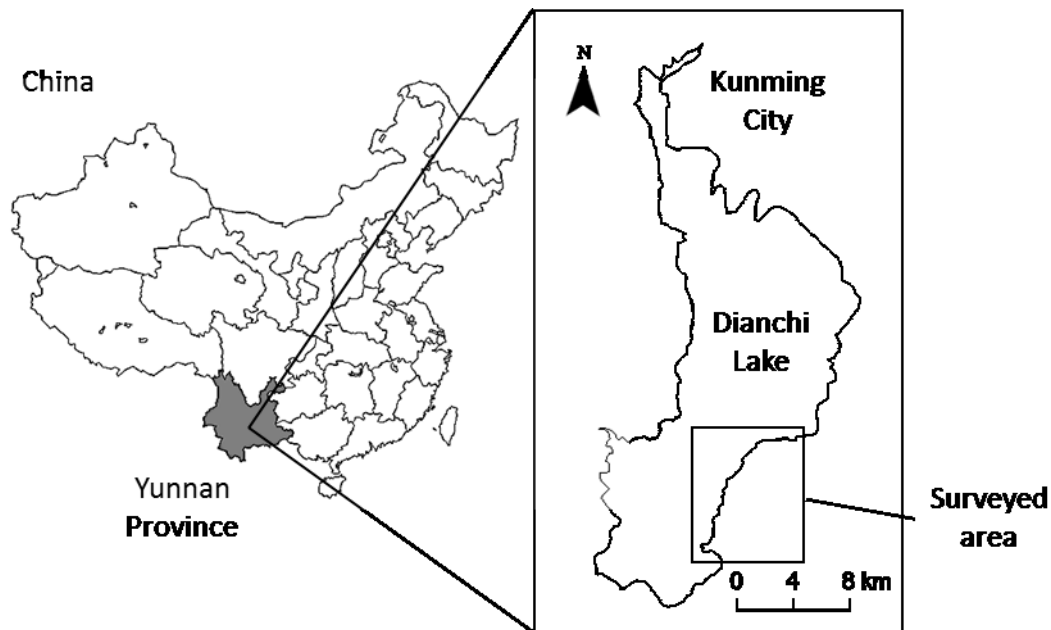


Figure 1.1 Locations of Dianchi Lake and the survey area.

1.3 Agricultural intensification in the southeastern basin of Dianchi Lake

The southeastern shore of Dianchi Lake (Figure 1.1) is a suburban supply base for agricultural and livestock products for Kunming City, where the residential population is 6,280,000 (Wang *et al.* 2015). Since the early 2000s, traditional paddy fields have been replaced by intensive cultivation of vegetables and flowers using greenhouses to meet increasing demands from the Kunming urban area. Accordingly, large amounts of chemical fertilizers and animal manure are applied within the area to achieve high yields. Around Kunming City, paddy fields have decreased by 45%, while vegetable fields have increased by 153%. In addition, the consumption of chemical fertilizers increased by 44% between 1999 and 2010 (Moritsuka *et al.* 2013; Statistics Bureau of Yunnan Province 2000, 2011). Intensification of animal production has also occurred by increasing the

amounts of feed purchased outside the area. Therefore, animal production is suspected to contribute to the increased nutrient load on croplands via manure application.

In association with imbalances in nutrient budgets, another problem in this area may be of concern for dairy production. Because several vegetable and flower species have a tendency to uptake and deposit nitrates from the soil (Pietro 2006), some plants in the area might have high nitrate levels resulting from heavy application of chemical fertilizers and spreading of animal manure. The residues and market irregulars from the vegetables and flower crops are supplied to dairy cows in the area. Therefore, the dairy cows may be ingesting excessive quantities of nitrates and may be affected by nitrate poisoning.

1.4 Approaches for nutrient management

To correct nutrient imbalances and associated problems, nutrient budget approaches have been developed in agroecosystems for more than a century (Oenema *et al.* 2003). Oenema *et al.* (2003) distinguished three main purposes of nutrient budget studies: (i) to increase the understanding of nutrient flows, (ii) as performance indicators and awareness raisers for nutrient management, and (iii) as regulatory policy instruments. To achieve these purposes, various approaches have been attempted and developed in previous nutrient budget studies. Within the context of an agroecosystem, the approaches were grouped into three types: farm-gate balances, soil surface balances (or field balances) and systems balances (Öborn *et al.* 2003).

The simplest approach is the farm-gate balance in which nutrient inputs and outputs beyond a farm-gate or a system boundary are calculated. The nutrient surplus, the

difference between the inputs and outputs, can be a potential nutrient load to the environment. The nutrient use efficiency, the ratio of the outputs divided by the inputs, serves as an indicator of efficient nutrient use. Because the farm-gate balance is relatively easy to calculate, the nutrient surplus and use efficiency have often been adopted as nutrient balance indices by policy makers. For example, MINAS, the Dutch MINeral Accounting System, regulates and imposes fines on annual N and P surpluses per area of farmland to mitigate the eutrophication of the regional aquatic systems (Schröder & Neeteson 2008). Fangueiro *et al.* (2008) reported that the farm-gate N surpluses per area of farmland in dairy farms were positively correlated with the intensity of farming, but were negatively correlated with the surpluses per milk production and with the use efficiency. However, the P and K surpluses per area did not have a significant correlation with farming intensity. Because the farm-gate approach cannot reveal internal nutrient flows within the system, it is difficult to identify the causes of the surplus or the pathways and amounts lost to the environment.

The soil surface balance approach quantifies nutrient inputs to soils via the surface and the output by crop uptake. The soil surface surplus can be used as a more direct indicator of the nutrient loss from farmland soil than the farm-gate nutrient surplus. The soil surface surplus has been used as an environmental index by OECD (OECD 2001). This approach makes it easy to visualize the spatial pattern of the nutrient budget or load. Using a mapping approach, the hot spots of the environmental pollution load can be visually understood. Bouwman *et al.* (2013) described global agricultural soil N budgets over the period 1900-2050 on a world map. The results showed a rapid increase in the surplus for North America and a further increase for northwestern Europe and South and

East Asia. However, this approach cannot distinguish between internal and external nutrient flows in the production system which includes several subsystems.

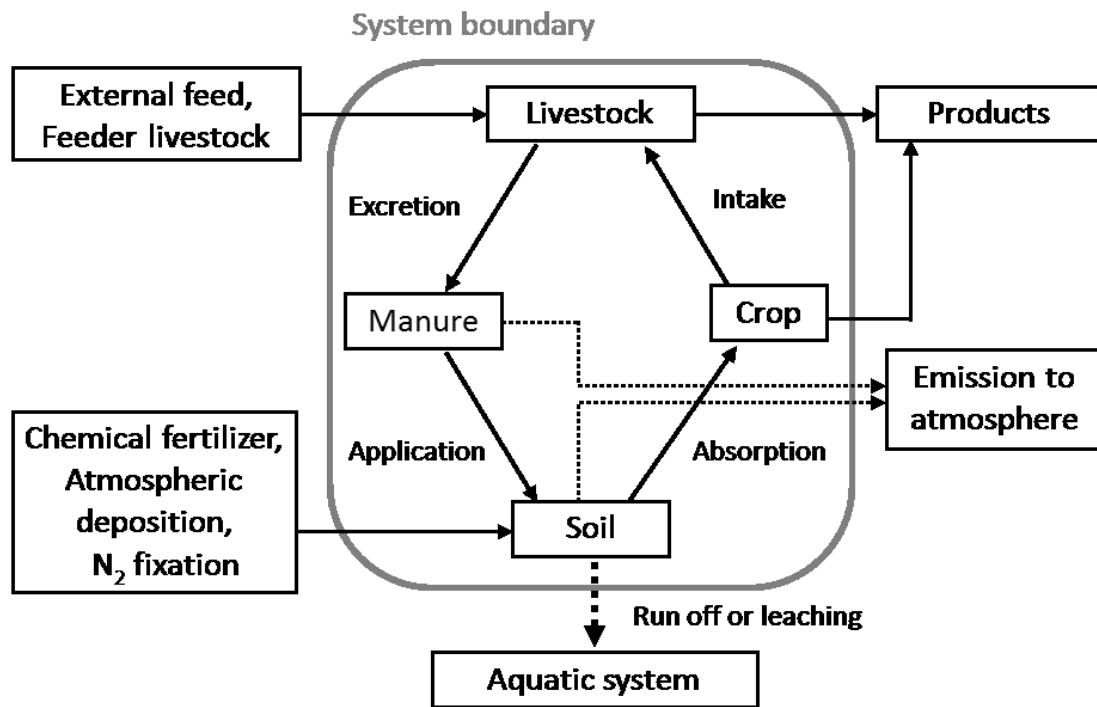


Figure 1.2 Nitrogen flow in an agricultural system.

To understand the internal nutrient flows within a system, the systems balance approach is most desirable. This approach provides information on the partitioning of the changes in net loading between system components (Öborn *et al.* 2003). The system boundaries can be set from farm scale to regional or national scales. The flow of N in a typical agricultural system is shown in Figure 1.2 as an example of the nutrient flow in agroecosystems. As shown in the figure, although surplus N is leached or run off through the farmland soil, N is cycled between animal and crop production subsystems in the form of feed and manure. Consequently, animal production indirectly contributes to N leaching from soil to water; a similar pattern is observed with other nutrients, such as P and K.

Therefore, animal production is one of the key drivers in nutrient cycling within the agricultural system as a whole, and appropriate nutrient management is required for both crop and animal production subsystems for efficient nutrient use throughout the agricultural production system. Although the systems balance approach requires detailed information on all flows within a system, the subsystems causing an imbalance in the nutrient budget can be identified using this method.

In addition to the indicators of nutrient utilization efficiency and surplus, nutrient cycles within a system can be evaluated by calculating a cycling index in the systems balance approach. The concept of the cycling index was developed in the field of ecology (Finn 1980) and introduced to nutrient cycle assessment in agroecosystems (e.g., Tabata *et al.* 2009; Rufino *et al.* 2009). The cycling index represents the ratio of nutrient cycling to the total nutrient flow in the system and is defined as the ratio of the cycled portion to the total system throughflow (Finn 1980). The cycling index is zero when no nutrients are cycled and one when nutrients are completely cycled (Tabata *et al.* 2009). As system components increase, calculation of the cycling index becomes more complex. Kimura and Hatano (2007) simplified the calculation of the cycling index and created additional indices, including the proportion of export and loss to total system throughflow, export and loss indices.

The nutrient budget studies have been conducted based on small-scale analyses in farms, which included experimental procedures such as chemical analyses of feed composition (e.g., Aarts *et al.* 2000a,b; Steinshamn *et al.* 2004), and on large-scale surveys covering entire regions or countries using census or statistical data (e.g., Hatano *et al.* 2002; Bouwman *et al.* 2011). Although detailed nutrient flows could be understood by analyses at a farm level, results would be difficult to expand to regional scales due to

the differences in feeding and management practices between farms. However, large-scale surveys tend to result in rough estimations, especially for animal production because these surveys often depended on basic units of nutrient budgets per head quoted from previous studies. In this thesis, therefore, we attempted to estimate nutrient flows in animal production at a regional scale using the procedures detailed below (Figure 1.3). First, the local feeding and management practices were standardized for dairy cattle, fattening pigs, breeding sows, broilers and laying hens by multiple interview surveys of several farmers. Feed intakes were measured at the farms or estimated using feeding standards; sampled feeds were analyzed for their chemical composition. Using these data, nutrient budgets were calculated at an individual level by animal category. The budgets calculated were expanded to the flows at the regional scale in combination with information on manure destination, feed production area and the number of animals obtained from field surveys.

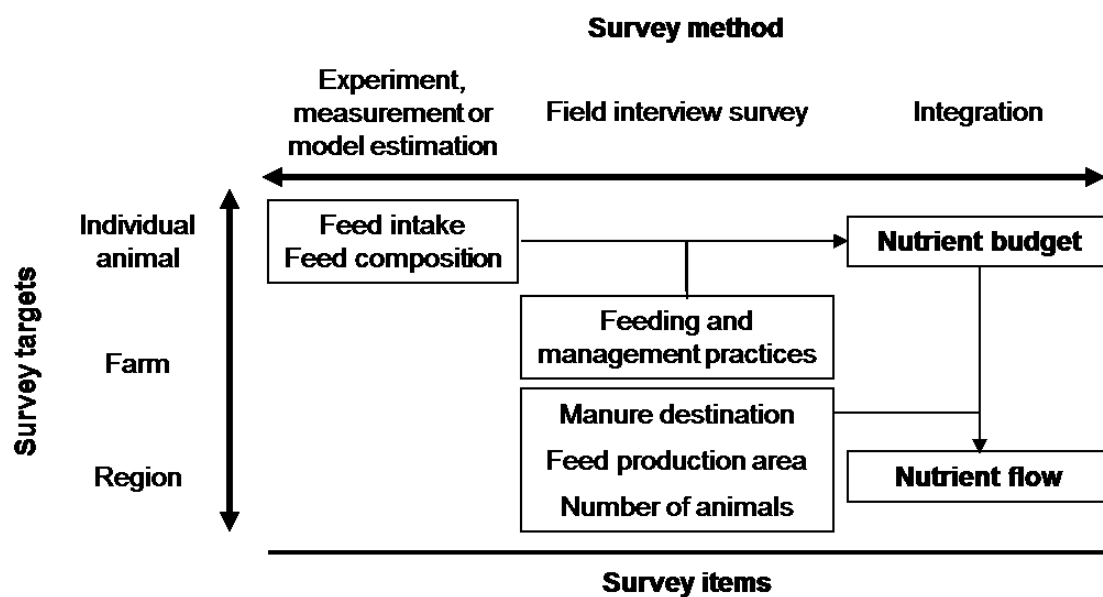


Figure 1.3 Flow diagram of the nutrient flow estimation procedure used in this thesis.

1.5 Objective

The objective of this thesis was to evaluate nutrient flows in animal production in the southeastern basin of Dianchi Lake, where nutrient budget imbalances were suspected. To meet this objective, three studies were conducted as outlined here.

Chapter 2 describes the effect of animal production on flows for N and P, a major contributor to water eutrophication. The N and P budgets for each animal production system (dairy cattle, fattening pigs, breeding sows, broilers and laying hens) in the area were calculated, and subsequently, the effects of the entire animal production on N and P flows in the agricultural system were evaluated. In Chapter 3, K and magnesium (Mg) budgets and flows for animal production were calculated and evaluated as well as N and P in the Chapter 2. Because dietary K can affect urinary N excretion in animals (Kojima *et al.* 2005) and Mg in manure can affect the release of P from soils (Josan *et al.* 2005),

these elements were considered to indirectly contribute to N and P discharged from agricultural production systems. However, because K and Mg are currently not environmentally regulated, these compounds are often routinely overfed, have low absorption efficiency and may be excreted in large quantities in animal manure (Hristov *et al.* 2007). In Chapter 4, dietary nitrate loads on the dairy cows were quantified by analyzing nitrate contents in feedstuffs and determining methemoglobin levels in the blood of cows. Nitrate poisoning was suspected to be occurring in dairy cows in association with imbalances in nutrient budgets, as mentioned above. In Chapter 5, regional nutrient cycling, exports and losses in the agricultural production system including animal and crop production were evaluated and discussed in combination with the research outcomes from the nutrient balance analyses of crop production in the area.

CHAPTER 2

Estimation of nitrogen and phosphorus flows in animal production in the southeastern basin of Dianchi Lake

2.1 Introduction

Nitrogen (N) and phosphorus (P) are essential nutrient elements in both crop and animal production within agricultural production systems. Over the past several decades, crop yield per unit area and animal production per animal have been greatly improved by agricultural intensification, in which N and P inputs into agricultural systems have drastically increased through the use of chemical fertilizers for crop production and concentrate feed for animal production. Parts of the N and P inputs were not used in the production systems and thus the excessive N and P surplus were emitted into the environment, causing significant environmental problems, such as eutrophication and high nitrate concentrations.

A nutrient budget approach has been developed to quantify the impact of nutrients emitted from agricultural systems into the environment. In this approach, the flows of a nutrient are estimated by calculating the inputs and outputs of systems (Öborn *et al.* 2003) and nutrient surplus (i.e., the difference between nutrient inputs and outputs) represents the potential effect on the environment. With respect to N, the N surplus in agricultural fields has a high correlation with the amount of N leached from the land (Barry *et al.* 1993; Goss & Goorahoo 1995; Hayashi & Hatano 1999). With regard to P, an excess assimilation of P in the soil causes exponential P leaching (Hechratch *et al.* 1995) and thereby facilitates eutrophication (Mishima *et al.* 2003).

To solve such environmental problems, various studies have been conducted from small-scale detailed dynamic nutrient analyses in experimental farms (e.g., Aarts *et al.* 2000a,b; Steinshamn *et al.* 2004) to large-scale surveys covering entire regions or countries (e.g., Hatano *et al.* 2002; Bouwman *et al.* 2011). Farmers and policy makers

have also used the nutrient budgeting approach for their nutrient management at farm to country scales (Oenema 2003).

In China, environmental problems caused by imbalances in nutrient budgets have become quite serious due to the rapid intensification of agriculture. The annual amounts of chemical fertilizer applied in China in 2010 were 3.5×10^7 t N and 1.7×10^7 t P_2O_5 , the largest amounts in the world, accounting for approx. one-third of the total global use (FAO 2012). Thus, the environmental impacts from Chinese agricultural systems could be significant on a global scale.

Dianchi Lake, located in the south of Kunming, the capital of Yunnan Province, is the sixth largest lake in China and is an essential water source for the livelihood of thousands of local residents. This lake has been seriously polluted due to the development of a catchment area beginning in the 1970s. In the southeastern shore areas of the lake, traditional paddy fields have been replaced by intensive cultivation of vegetables and flowers, using greenhouses to meet increasing demands from the nearby Kunming urban area. Accordingly, large amounts of chemical fertilizer and animal manure are applied in the area in order to achieve high yields. As an example of conventional fertilization, the amounts of N and P for celery were 4,800 and 1,571 kg/ha/yr in this area (Guo *et al.* 2006).

In fact, by analyzing soil samples from greenhouses, uplands and paddy fields in the area, Moritsuka *et al.* (2013) indicated that soluble nutrients, especially inorganic N, were highly assimilated in the greenhouse soil due to over-application of fertilizers and thereby the amounts of readily available N and P in the greenhouse soils exceeded 5,000 kg/ha. In addition, following their analyses of surface water samples in rivers that flow into Dianchi Lake from the area, Tanaka *et al.* (2013) reported that N (NO_3 -N and Total N)

pollution in the rivers was extremely high in the rainy season, and cumulative P loads were observed in the lower reaches of the rivers.

The animal production in the area has been highly intensified and is suspected to indirectly contribute to the N and P loads on the greenhouse soil and the water pollution, by the increasing input of concentrate feeds from outside the area and the large amounts of application of manure within the area. The objectives of the present study were to calculate N and P budgets for each animal category (dairy cattle, fattening pigs, breeding sows, broilers and laying hens), and to evaluate the effects of the entire animal production system on N and P flows in the southeastern basin of Dianchi Lake.

2.2 Materials and methods

Site description

The investigation was conducted in the cropland area near the southeastern shore of Dianchi Lake (24.7°N, 102.7°E, 1,892 m above sea level), Kunming City, Yunnan Province, China (Fig. 2.1). Because of its high altitude, this low-latitude area has been classified as a temperate monsoon climate, and the annual mean temperature and annual precipitation from 2009 to 2013 were 16.2°C and 740 mm, respectively (Japan Meteorological Agency 2014). The area targeted in this study consisted of 27 administrative villages within a distance of 40 km from the city center of Kunming. The total area, population and number of households were 46.8km², 53,677 and 17,714, respectively.

The agricultural characteristics of the study area obtained from the village offices in 2009 are shown in Table 2.1. The cultivation of vegetables and flowers was very popular in the area, and most of these crops were grown in greenhouses. Approximately 60% of the total area was cultivated, and 85% of the cultivated area was covered with greenhouses. Dairy cattle, pigs and chickens were kept in the area. Very few animal farmers had meadows or farmlands for feed production, and the majority purchased feeds and sold manure.

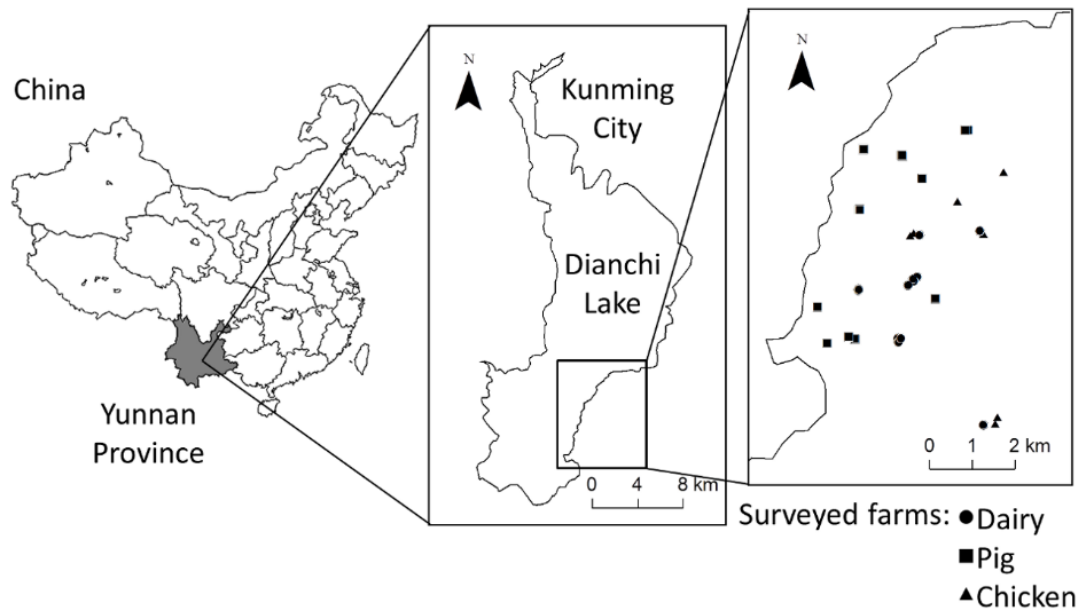


Figure 2.1 Locations of the study site and surveyed farms.

Table 2.1 Agricultural characteristics of the study area in 2009

Cultivated type	Land area (ha)	Species	No. of animals (head)
Grain	167	Cattle	3,637
Vegetable	1,664	Pigs	32,025
Flower	895	Chickens	217,144
Other	73		
Total	2,799		

Field survey

We conducted field surveys on the animal farms eight times from October 2009 to December 2011. In the surveys, we visited 11 dairy farms, seven breeding sow farms, six fattening pig farms, four laying hen farms, four broiler farms and nine feed dealers. The duration of each survey was approx. 1 wk.

The surveys included interviews and feed samplings. The interviews contained questions on feed composition, the amount of feed offered, animal production (milk, meat and egg yield) and reproductive performance (litter size and breeding cycle), management (e.g., feeding period and body weight at the time of purchase and sale), and manure management. The production area of each feed was determined by asking local animal farmers and feed dealers. The information obtained was validated by revisiting the same farmers on several occasions.

On the dairy farms, all types of the feed offered were sampled and the amount of each feed on an as-fed basis was weighed at the sites. The feed samples were dried at 70°C for 48 h, and the dry matter (DM) contents were measured immediately.

Nitrogen and phosphorus contents of feeds

The N and P contents of some of the feeds for cattle and pigs were obtained by chemical analyses, and those of the other feeds were obtained from the published Feeding Standards of Chickens, Dairy Cattle and Swine (Ministry of Agriculture of the People's Republic of China 2004a,b,c).

The feed samples were ground using a Wiley mill for the chemical analyses. The total N contents of the feed samples were calculated by adding the nitrate N contents

analyzed by the Cataldo method (Cataldo *et al.* 1975) to the Kjeldahl N contents in the samples measured by the Kjeldahl method (AOAC 2000).

Phosphorus contents in the samples were determined by the colorimetric method (Gomori 1942) using a spectrophotometer (UV-160; Shimadzu, Kyoto, Japan), after wet ashing with nitric and perchloric acid.

Key information for the calculation of the N and P budgets

For the calculation of N and P budgets for each animal category, the amounts of feed intake, feed composition, animal production and reproductive performance and the feeding periods for each animal production system were specified according to information obtained from the interviews of farmers. When accurate information on feed intake was not available, the intakes were estimated using equations given in feeding standards. It was noted that, in this case, the estimated feed intake was assumed to be equal to the requirement.

Dairy cattle

We evaluated the N and P flows for a lifetime of one Holstein cow, which was the sole breed in the area. A heifer was grown for 2 yrs from 40 kg to 400 kg. After maturation, six calves (40 kg each) were delivered to the cow's age of 8 yrs (body weight [BW] 563 kg). It was assumed that five calves were sold immediately after their births and one calf was kept for replacement to maintain the herd size. The annual milk yield and lactating period were set at 6,590 kg/yr and 305 days/yr, respectively. The intake in lactation periods, dry matter content, N and P contents, and the production area of each feed are shown in Table 2.2. Fermented corn residue, corn grain, soybean curd residue, rice bran

and wheat flour were fed as concentrates, and corn stem silage, rice straw and broad-bean stems were fed as roughage. Local vegetable residue was also used as roughage in large amounts. The residues of many types of vegetables and flowers including broccoli, celery, parsley, stem lettuce, purple cabbage, rape blossoms and carnations were seasonally available. The amounts of these residues fed to dairy cattle were 28.4 to 50.0 kg per head per day in fresh weight in the dairy farms, which accounted for 25% to 31% of the total feed on a dry weight basis.

Since most of the dairy farmers in the area did not have their own land for feed crops, the vegetable and flower residues from an area nearby were preferable in terms of cheap price and high availability compared to feeds purchased far from their farms. To the best of our knowledge, there have been no previous reports regarding a dairy cattle diet with large amounts of fresh vegetables and/or flowers. Although the types of vegetable residues differed from day to day, broccoli and Chinese cabbage residues were fed most frequently during the surveyed periods. Therefore, “vegetable residues” were assumed to be evenly halved broccoli and Chinese cabbage residues at a fresh weight basis in the evaluation. The feed intakes during the growing and non-lactating periods were estimated using the equations of DM requirement obtained from the Japanese Feeding Standard for Dairy Cattle (NARO 2006), and the feed compositions were considered to be the same as those during lactating periods.

Table 2.2 Feed intake and composition used in the calculation of N and P budgets for dairy cattle

	Feed intake (kg/day)	DM (%)	N (%DM)	P (%DM)	Production area
Fermented corn residue	9.0	22.9	3.3	0.38	Local
Corn grain	6.3	88.4	1.2	0.23	External
Soybean curd residue ¹	2.5	10.1	4.9	0.30	Local
Rice bran ¹	1.0	90.2	2.1	1.15	External
Wheat flour ¹	0.3	87.8	2.0	0.15	External
Corn stem silage	4.2	22.7	1.4	0.17	External
Rice straw	2.1	95.7	0.8	0.12	External
Broad bean stem	1.5	84.9	1.7	0.12	Local
Vegetable residue ²	46.5	7.8	4.7	0.46	Local
Total	73.3	22.9	2.3	0.32	—

¹Dry matter, N and P contents were obtained from the Ministry of Agriculture of the People's Republic of China (2004b).

²Vegetable residue was assumed to be evenly halved broccoli and Chinese cabbage wastes at a fresh weight basis.

Fattening pigs

A pig was fattened from 20 kg at the age of 2 months of introduction to 160 kg at the age of 8 months of finishing. Four of the six farmers interviewed produced sugar or starch from rice, and they provided its by-products (rice molasses) to their pigs. In addition to the molasses, corn grain and rice bran were fed in fixed proportions. The feed composition and the contents of DM, N and P of each feed are shown in Table 2.3. The total feed intakes were estimated from the digestible energy (DE) requirement using the Japanese Feeding Standard for Swine (NARO 2005).

Table 2.3 Feed composition used in the calculation of N and P budgets for fattening pigs and breeding sows

	Ratio(%) ¹	DM(%)	N(%DM)	P(%DM)
<i>Fattening pigs</i>				
Rice molasses ²	69.2	22.9	5.3	0.70
Corn grain	23.1	88.4	1.2	0.23
Rice bran ²	7.7	87.0	2.4	1.64
Total	100.0	42.9	2.9	0.63
<i>Breeding sows</i>				
Corn grain	60.0	88.4	1.2	0.23
Soybean meal ²	20.0	89.0	8.6	0.73
Wheat bran ²	10.0	87.0	2.9	1.06
Fish meal ²	6.0	90.0	11.1	3.39
Vitamin supplement ³	4.0	90.0	–	1.00
Total	100.0	88.5	3.4	0.63

¹Fresh weight ratio in the total feed.

²Dry matter, N and P contents were obtained from the Ministry of Agriculture of the People's Republic of China (2004c).

³Dry matter, N and P contents were obtained from industrial data.

Breeding sows

Budgets were calculated for a breeding sow and its piglets. A sow was purchased at the weight of 130 kg and culled at 230 kg after the sixth farrow. One breeding cycle was set at 143 days, with pregnancy, suckling and return of estrus periods of 115, 21 and 7 days, respectively. Litter size was set at 12 heads (1.4 kg/head), and the lactation during the suckling period was 7.5 L/day. The feeds for the sows were corn grain, soybean meal, wheat bran, fish meal and vitamin supplements. The feed composition and the contents of DM, N and P of each feed are shown in Table 2.3. The total feed intakes from the DE

requirement were estimated using the Japanese Feeding Standard for Swine (NARO 2005).

Piglets were grown for 60 days and fed starter feed along with suckling. The amounts of N and P intake were assumed to be equivalent to N and P requirements obtained from the Feeding Standards of Swine (Ministry of Agriculture of the People's Republic of China 2004c). All of the feeds for the sows and piglets were commercially processed and packaged and were imported from outside the area.

Broilers and laying hens

A broiler was grown from 25 g at hatching to 3 kg at the age of 56 days of finishing, and a laying hen was fed from 20 g at hatching to 2 kg at the age of 450 days. Egg production began at the age of 130 days, and the lifetime egg yield of a hen was set at 220 eggs (61 g/egg). Only feed mixture purchased from outside the area was used for both types of chickens. The feed intake for each week was derived from the Japanese Feeding Standard for Poultry (NARO 2011). The intake and N and P contents of feed at each growth stage for broilers and laying hens are shown in Table 2.4.

Table 2.4 Feed intake and composition used in the calculation of N and P budgets for broilers and laying hens

Age (wks)	Feed intake (g/day)	N (%)	P (%)
<i>Broilers</i>			
1 to 3	52.6	3.44	0.68
4 to 6	145.4	3.20	0.65
7 to 8	189.6	2.88	0.60
<i>Laying hens</i>			
1 to 8	29.5	3.04	0.70
9 to 19	70.1	2.48	0.60
20 to 64	120.0	2.56	0.60

Dry matter, N and P contents were obtained from the Ministry of Agriculture of the People's Republic of China (2004a).

Framework of the nutrient flow system

In this study, N and P flows based on the “systems balances” approach were calculated based on the nutrient cycling between animal and crop production systems within the area. This approach is the most detailed among the types of element balance analyses, and it provides information on the partitioning of the changes in net loading between system components (Öborn *et al.* 2003). The framework of the nutrient flows in the present study is shown in Figure 2.2. Only the flows relevant to animal production were assessed in this study. The system boundary was set around the whole animal production in the study area. Two types of feeds were distinguished based on their production areas; feeds produced within the area were defined as ‘local feed’, and those imported from outside the area were defined as ‘external feed’. All nutrients contained in the livestock products (including retained nutrients in animals) were exported outside the system.

With respect to manure management, the animal excretion was collected without separation into urine and feces, and applied to cropland without enough composting. The farmers who had lands and cultivated crops applied at least manure from their own farm to their own lands. However, since most of the animal farmers in the area had no farmlands for feed crops, they sold large amounts of unused manure to neighboring crop farmers or manure dealers. Therefore, manure produced by the local farms was assumed to be applied to crop fields in the area. As N in excretions is partly lost by ammonia volatilization in housing and storage before spreading, the ammonia emission factor was set at 10% for dairy cattle, 20% for pigs, and 30% for poultries according to the National Greenhouse Gas Inventory Report of Japan (Greenhouse Gas Inventory Office of Japan *et al.* 2014). Phosphorus loss before spreading manure to the croplands was not considered.

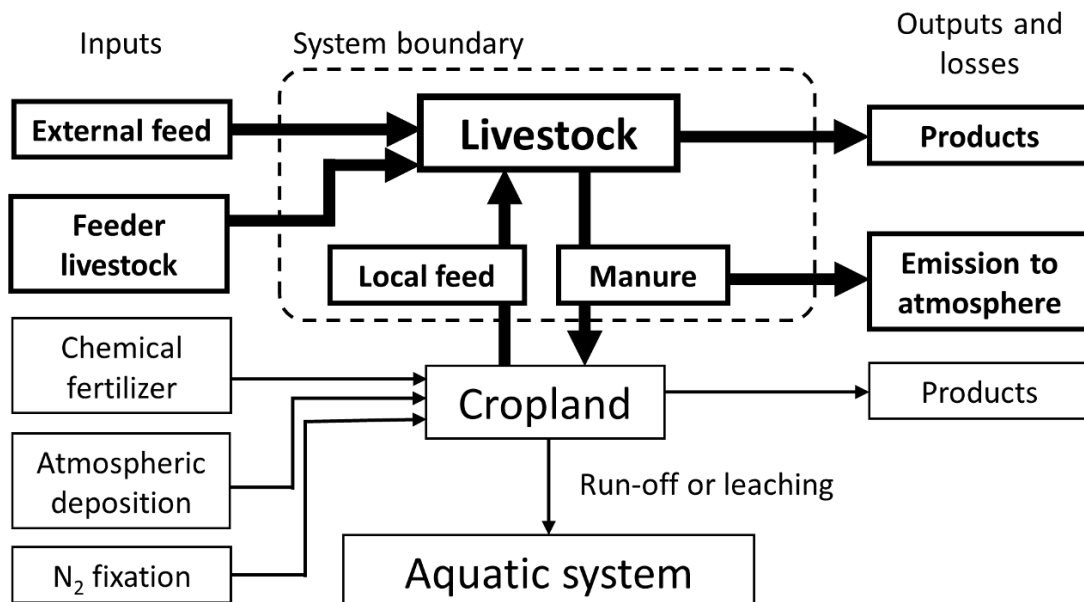


Figure 2.2 Framework of the nutrient flow in the agricultural systems. Flows indicated by boldface were estimated in the present study.

Calculation of the N and P budgets at the individual level

We calculated the intakes of N and P by multiplying the N and P contents of each feedstuff by the amounts of intake and then summing them up (Eq. 1). Feed intake was assumed to be the same as the amount offered because of very little refusals.

$$N \cdot P_{\text{intake}} (\text{g/day}) = \sum_i (\text{Amount of feed } i (\text{kg/day}) \times N \cdot P \text{ content of feed } i (\text{g/kg})) \quad (1)$$

The amounts of N and P retained in the animals and those in milk and eggs were calculated by multiplying the amounts of body weight gain, milk and eggs by the N and P contents per unit of body weight gain and those of milk and egg, respectively (Eqs. 2, 3).

$$\begin{aligned} N \cdot P_{\text{retention}} (\text{g/day}) \\ = \text{Daily gain (kg/day)} \times N \cdot P \text{ content per unit of body weight gain (g/kg)} \end{aligned} \quad (2)$$

$$\begin{aligned} N \cdot P_{\text{milk}\cdot\text{egg}} (\text{g/day}) \\ = \text{Milk or egg yield (kg/day)} \times N \cdot P \text{ content of milk or egg (g/kg)} \end{aligned} \quad (3)$$

The N and P contents of each product used in the calculations are shown in Table 2.5.

The amounts of N and P excreted were calculated by subtracting the amounts of retained N and P and the amounts of N and P in milk and eggs from the amounts of N and P intake (Eq. 4).

$$N \cdot P_{\text{excretion}} (\text{g/day}) = N \cdot P_{\text{intake}} (\text{g/day}) - N \cdot P_{\text{milk}\cdot\text{egg}} (\text{g/day}) - N \cdot P_{\text{retention}} (\text{g/day}) \quad (4)$$

Table 2.5 N and P contents in animal products (g/kg) used in the calculation of N and P budgets

Item	N	P	Reference
Cow milk	5.2	0.9	MEXT (2005)
Egg (with shell)	19.0	5.4	Watanabe (1996)
Cattle body	25.0	8.0	ARC (1980)
Fattening pig body	23.0	5.0	De Boer <i>et al.</i> (1997)
Breeding sow body	26.2	4.0	De Boer <i>et al.</i> (1997)
Newborn piglet body	19.2	6.2	De Boer <i>et al.</i> (1997)
Piglet body at 20 kg	24.0	5.1	De Boer <i>et al.</i> (1997)
Broiler body	28.0	4.7	De Boer <i>et al.</i> (2000)
Young layer body	28.0	6.3	De Boer <i>et al.</i> (2000)
Laying hen body	28.0	3.1	De Boer <i>et al.</i> (2000)

Estimation of N and P flows in the animal production system

We estimated the N and P flows in the overall animal production system in the area by multiplying individual N and P budgets by the number of each type of animal in the area (Table 2.1). Because beef production was less popular in the area and feeding purposes (e.g., fattening and breeding) were not classified in the statistical data in the area, all ‘cattle’ in the table were assumed to be dairy cattle. Supposing that the supply-demand balance of piglets was maintained within the area, the proportions of sows, piglets and fattening pigs were estimated to be 4.7%, 23.8% and 71.4% of the total number in the category of ‘pigs,’ respectively. The numbers of broilers and laying hens were both estimated to be one-half of the number of ‘chickens.’

2.3 Results and discussion

N and P budgets at the individual level

The N and P budgets for each animal category are shown in Table 2.6. In the dairy cattle and fattening pigs, N and P supplied from local crops or by-products accounted for large parts of the inputs in the production systems, whereas most of the N and P in feed depended on external resources in other animal categories. The detailed explanations for each animal category are described in the following subsections.

Dairy cattle

Of the total feed nutrients, 70% of the N and 49% of the P originated from local feed. In the case of N and P cycling in Dutch commercial dairy farms (Aarts *et al.* 2000a,b), 65% of the N and 57% of the P out of the total feed nutrients were from within the farms. The proportions of local feed N and P in the present study were comparable to the values given by Aarts *et al.* (2000a,b). This was largely due to the use of residues from vegetables and flowers cultivated in the local cropland as feed, which accounted for 44% of the N and 31% of the P in the total feed. These results indicate the importance of these residues in the local nutrient cycle for dairy production; 67% of the N and 65% of the P were converted to manure.

Table 2.6 Calculated N and P budgets for each animal category (kg/head/yr)

	Dairy cattle		Fattening pigs		Breeding sows		Broilers		Laying hens	
	N	P	N	P	N	P	N	P	N	P
Feeder livestock	–	–	0.95	0.20	1.45	0.22	0.00	0.00	0.00	0.00
Total feed	110.69	15.30	25.89	5.58	63.05	12.49	1.38	0.28	0.94	0.22
Local feed ¹	77.05	7.53	17.49	2.30	–	–	–	–	–	–
External feed ²	33.63	7.76	8.40	3.28	63.05	12.49	1.38	0.28	0.94	0.22
Production	27.95	5.36	7.27	1.58	17.27	3.52	0.55	0.09	0.25	0.06
Excretion	82.74	9.94	19.57	4.20	47.24	9.19	0.84	0.19	0.69	0.16
Manure	74.46	9.94	15.66	4.20	37.79	9.19	0.59	0.19	0.48	0.16
NH ₃ volatilization	8.27	–	3.91	–	9.45	–	0.25	–	0.21	–
Use efficiency (%) ³	25.3	35.0	24.4	24.7	25.1	26.4	39.3	32.4	26.8	28.9

¹Fermented corn residue, soybean curd residue, broad bean stem, and vegetable residue for dairy cows and rice molasses for fattening pigs.

²Feeds except local feed.

³(Production-Feeder livestock)/Total feed*100.

The average daily N intake at lactation periods was 386 g. The N intake estimated in the present study was 17% higher than the daily N requirement for lactating cows (body weight, 550 kg; daily milk yield, 21.6 kg; and milk fat, 4%) calculated by the Japanese Feeding Standard for Dairy Cattle (NARO 2006). The feed-to-milk N use efficiency (feed-NUE: the proportion of N in milk out of N consumed as feed by lactating cows) was 28.4% in the present study, which was within the range encompassing most on-farm feed-NUE, according to Chase (2003). The average excreted N during lactation periods was measured at 277 g/day in the present study, which was 9% lower than the estimation for Japanese lactating cows (BW, 650 kg; daily milk yield, 25 kg) by Tsuiki and Harada (1997).

The total diet P content was 0.32% DM, which was approximately equal to the total diet P requirement for lactating cattle (BW, 600 kg; daily milk yield, 20 kg) according to the Japanese Feeding Standard for Dairy Cattle (NARO 2006). Excreted P during lactation periods was measured at 32.8 g/day in the present study, which was 25% lower than the estimation by Tsuiki and Harada (1997).

Fattening pigs

In the total feed nutrients, 68% of the N and 41% of the P originated from local feed. In addition to vegetable residues for dairy production, rice molasses played an important role in the local nutrient cycling for fattening pig production. Sixty percent of the N and 75% of the P were converted to manure.

According to the Japanese Feeding Standard for Swine (NARO 2005), the N requirements for growing pigs (BW from 30 to 70 kg) and finishing pigs (from 70 to 115 kg) are 51.8 g/day and 65.0 g/day, respectively. The average N intakes for the pigs in the

present study were 51.3 g/day and 76.8 g/day, providing 99% and 118% of the requirements, respectively. The average excreted N for fattening pigs in the present study was 53.6 g/day, which is 57% higher than the estimation (34.2 g/day) by Tsuiki and Harada (1997). This difference was partly explained by the difference in the body weight of pigs: the initial and final body weights in the study were assumed to be 20 kg and 160 kg, respectively, whereas Tsuiki and Harada assumed the weights 1 kg and 115 kg.

In the present study, the total diet P content for fattening pigs was 0.63% DM. Since pigs are unable to utilize phytin P, the P requirement for pigs is generally expressed as non-phytin P. Non-phytin P content in the diet was estimated to be 0.18% DM because of the low non-phytin contents of rice molasses and corn grain, which did not meet the requirement, 0.22% DM (NARO 2005). Excreted P in the present study was 11.5 g/day, which was 32% higher than the estimation (8.7 g/day) by Tsuiki and Harada (1997). Assuming that pigs utilized the all non-phytin P in the bodies, the P use efficiency would be up to only 28.6%. The P use efficiency could be increased by the use of higher non-phytin P-containing feed such as fish meal and the reduction of the total P content in the feed.

Breeding sows

The entire N and P in feed depended on 'external feed,' whereas 60% of the N and 74% of the P were converted from feed to manure and used in the area.

According to the Japanese Feeding Standard for Swine (NARO 2005), the N requirements for pregnant and lactating sows are 42.6 g/day and 128 g/day, respectively. The average N intakes for the pregnant and lactating sows in the present study were 62.5 g/day and 168 g/day, which were 147% and 130% of the requirements, respectively. The

average excreted N in the present study was 62.9 g/day, which was 23% higher than the estimation (51.0 g/day) by Tsuiki and Harada (1997). This difference might be due to the higher N intake than the requirement.

The total diet P content for breeding sows was 0.63% DM, with a non-phytin P content of 0.40% DM. The non-phytin P content in the diet for sows was almost double that for fattening pigs, and the total P contents were almost equivalent in the two diets. This was due to the use of fish meal, in which all P exists in the non-phytin form. However, the total diet non-phytin P content was lower than the requirement of 0.45% DM (NARO 2005). The excreted P in the present study was 11.4 g/day, which was 27% lower than the estimation by Tsuiki and Harada (1997).

Broilers and laying hens

As in the case of breeding sows, the entire N and P feed was 'external feed' for both broilers and laying hens. The proportions of N and P converted to manure were 42% and 68% for broilers and 51% and 71% for laying hens, respectively. The excreted amounts of N and P were 2.30 and 0.52 g/day for broilers and 1.88 and 0.43 g/day for laying hens, respectively.

N and P flows in the animal production at the regional scale

The estimated N and P flows in the animal production system in the area are shown in Table 2.7 and Figure 2.3. For overall animal production, 51% of the N and 31% of the P originated from within the area. Of the feed nutrients consumed by animals, 27% of the N and 28% of the P was converted to animal products, 60% of the N and 72% of the P was recycled within the area by the application of manure, and 14% of the N was lost

through the ammonia volatilization from the excretions. The N and P amounts imported from outside the area as the external feed exceeded the N and P exported outside the area as the animal products, and the N and P amounts of manure produced and applied in the area also exceeded the N and P used by the animals as local feed. These results implied that the animal production in this region potentially increased the N and P loads in the regional agricultural system. If pig and poultry productions use more local crop residues or other feed materials as well as dairy production, the proportion of external feed will be decreased, and nutrients could be used more effectively within the area.

The N and P amounts in manure were 805 tN/yr and 184 tP/yr, and those amounts divided by the area of the agricultural land in the area (2,799 ha) were 287 kgN/ha/yr and 66 kgP/ha/yr, respectively. In European Union (EU) countries, the application of manure to croplands has been restricted to below 170 kg/ha/yr of N by the Nitrate Directive in order to reduce nitrate levels in ground water (European Commission 2010), and the application actually ranged from 40 kg/ha of N in Spain to 180 kg/ha in Benelux countries (Oenema 2004).

To prevent eutrophication in the aquatic systems in The Netherlands, the P amount applied to cropland from animal manure and mineral fertilizer has been restricted to below 37 kg/ha/yr by the MINAS system (Schröder & Neeteson 2008). The estimated N and P amounts in the manure in the present study area exceeded these limitations. The comparison with EU regulations and situations indicates that the N and P loads by manure in the present study area were considerably high and should be reduced for the establishment of sustainable agricultural production systems in the future.

Livestock densities in the present study area were 77.7, 684 and 4,640 heads/km² for cattle, pig and poultry, respectively. According to the livestock density model developed

by Gerber *et al.* (2005), densities more than 500 heads/km² for pigs and 5,000 heads/km² for poultry were categorized into the highest levels in Asia and were observed only around urban centers. The livestock densities in the present study area were relatively high in Asia, and consequently the nutrient input for feed and the manure output also tended to be high. Hence, intensive efforts would be required for the effective use of manure and local feed nutrients in the broader region and the reduction of the livestock densities.

Table 2.7 Estimated N and P flows in animal production per area of agricultural land at the regional scale (kg/ha/yr)

	Cattle		Pig		Chicken		Total	
	N	P	N	P	N	P	N	P
Feeder livestock	–	–	0.8	0.1	0.2	0.0	1.0	0.2
Total feed	145.2	20.1	245.5	52.3	90.1	19.5	480.8	91.9
Local feed	101.1	9.9	143.1	18.9	–	–	244.2	28.7
External feed	44.1	10.2	102.4	33.5	90.1	19.5	236.6	63.2
Production	36.7	7.0	60.9	13.2	31.1	6.0	128.7	26.2
Excretion	108.6	13.0	185.3	39.3	59.2	13.5	353.1	65.8
Manure	97.7	13.0	148.3	39.3	41.4	13.5	287.4	65.8
NH ₃ volatilization	10.9	–	37.1	–	17.8	–	65.7	–

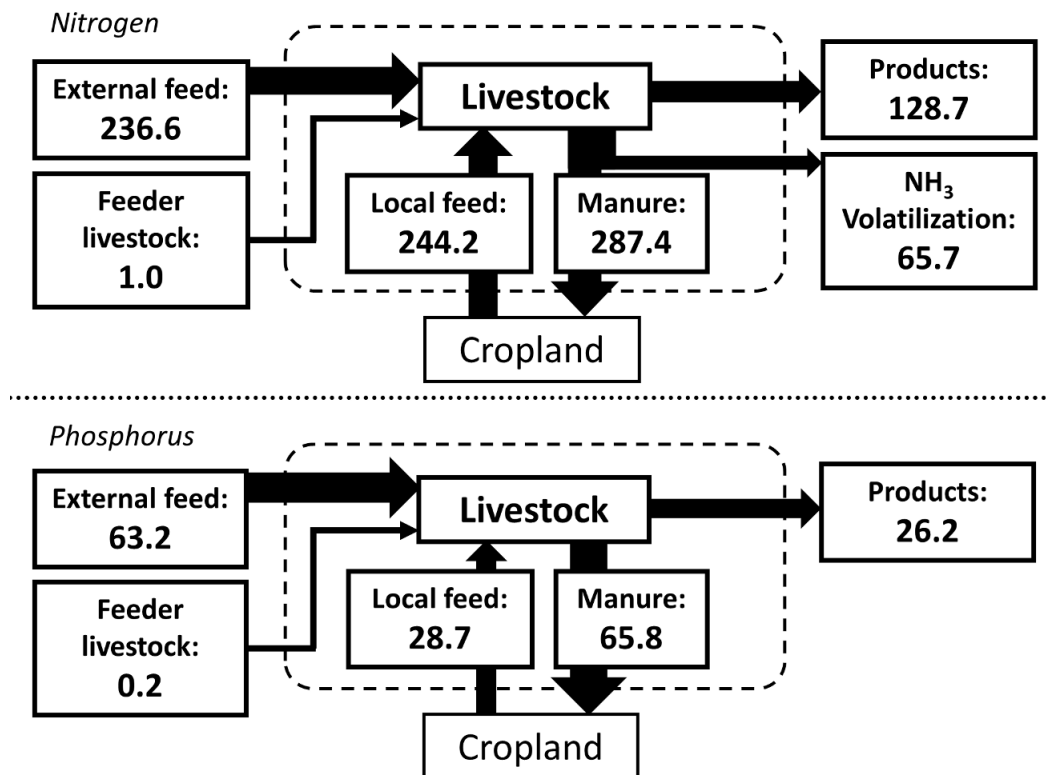


Figure 2.3 Nitrogen (upper) and phosphorus (lower) flows in animal production per area of agricultural land in the study area (kg/ha/yr). Width of arrows indicates each nutrient amount.

2.4 Concluding remarks

The results obtained from this study indicated that the animal production in the area contributed to the N and P loads on the croplands and consequently the aquatic systems. The detailed information on the N and P budgets for each animal category will be useful for farmers, who need to become familiar with the efficient use of nutrients in the feed of their animals. Moreover, the overall N and P flows estimated in this study can be used by

regional government entities and policy makers to devise a mitigation strategy against high N and P loads from animal production; e.g., restrictions on manure application and recommendations regarding the use of local feed resources.

The present study covered N and P flows related to animal production systems as the first step for resolving the water pollution problem of the Dianchi Lake. As the next step, information from other disciplines (e.g., crop science, soil science, horticulture, remote-sensing technology and agricultural economics) regarding the N and P flows of other agricultural production systems is required. The integration of such information will allow us to quantify N and P cycling in all of the agricultural production systems in the Dianchi Lake area and to predict N and P inflows to Dianchi Lake in the future.

CHAPTER 3

Estimation of potassium and magnesium flows in animal production in the southeastern basin of Dianchi Lake

3.1 Introduction

Nutrient losses in animal production have received increasing attention, and many studies have focused on the development of methods to evaluate and reduce the nutrient losses from farm-level to entire regional or country levels (Hatano *et al.* 2002; Fangueiro *et al.* 2008; Bouwman *et al.* 2011). Among the nutrients, nitrogen (N) and phosphorus (P) are focused on the most often due to their clearly adverse effects on the environment, including ammonia emission to the air, nitrate leaching into soil and ground water, and P leakage into surface and ground waters (Oishi *et al.* 2011). However, other elements that are currently not environmentally regulated are also routinely overfed or have low absorption efficiency and may be excreted in large quantities in animal manure (Hristov *et al.* 2007).

Potassium (K) and magnesium (Mg) are vital minerals in animals, and deficiencies of these minerals result in inappetence, ataxia, tetany and retarded of growth (NRC 1994, 2001, 2012). Excess K is usually excreted in urine by most domestic animals, and excess Mg is excreted in urine in ruminants (NRC 1980), suggesting that feeding excess K and Mg to animals increases their concentrations in excreta. In particular, the high K contents in home grown forages taken from K-rich soil due to excessive manure application to their land appear to be a problematic factor in the etiology of milk fever in dairy cattle and grass tetany in cattle (NARO 2008).

There is an inter-relationship between Mg and P; Mg can affect the P release in manure-amended soils (Hristov *et al.* 2007). Regarding manure-amended soil, Nair *et al.* (2003) and Josan *et al.* (2005) reported that the crystallization of the stable P form (Calcium-P) in sandy soil could be inhibited by the presence of a high level of Mg

resulting from intensive manure application. A reduction in the manure Mg concentration should thus be necessary to minimize the P release and the environmental impact of manure application if excessive P were applied to the soil.

Dianchi Lake, located in the south of Kunming City, Yunnan Province, China has received increasing attention since the 1970s because of its eutrophication. The coastal area of southeast Dianchi Lake is the biggest vegetable and flower supplier for Kunming City, and large amounts of chemical fertilizer and animal manure are applied in the area to increase the productivity of the farmland (Amachika *et al.* 2014). Moritsuka *et al.* (2013) investigated soil physicochemical properties in the area and reported that the average amounts of readily available N, P, and K accumulated in the greenhouse soil were estimated to be equal to or higher than the annual input of those as fertilizer. Wang *et al.* (2015), who described the annual nutrient balance in heavy multiple cropping systems in the area, also reported that the cropping intensity and input for each crop were extremely high, as 58%, 72% and 20% of N, P and K were not being absorbed by the crop in the vegetable field.

Together these findings revealed that the agricultural practices contributed to nutrient loads on the cropland in the area. The animal production in the Dianchi Lake area has been highly intensified, and the intensive animal production may also contribute to the nutrient loads on the cropland by the large amount of purchased feeds from outside the area and the excessive application of manure within the area.

The objectives of the present study were to calculate K and Mg budgets for animal production systems (dairy cattle, fattening pigs, breeding sows, broilers and laying hens), and to evaluate the effects of the animal production systems on K and Mg flows in the southeastern basin of Dianchi Lake on a regional level.

3.2 Materials and methods

Site description

The investigation was conducted in the cropland near the coastal area of the southeastern shore of Dianchi Lake, Kunming City, Yunnan Province, China. Twenty-seven administrative villages located in the coastal area were selected in the present study. The total area size was 46.8km², the population was 53,677 residents, and the number of households was 17,714. The agricultural characteristics of the study area obtained from the village offices in 2009 are shown in Table 2.1 in the previous chapter. In the coastal area of southeast Dianchi Lake, although the paddy rice-broad bean cropping system was dominant in the past, the cropping systems have been shifting to vegetable and flower production in greenhouses since the 2000s (Tanaka *et al.* 2013). Dairy cattle, pigs and chickens are kept in the area. Very few animal farmers have meadows or farmlands for feed production, and the majority of the animal farmers purchase feed and sell manure.

Field survey

Field surveys were conducted on the animal farms eight times from October 2009 to December 2011. In the surveys, 11 dairy farms, seven breeding sow farms, six fattening pig farms, four laying hen farms, four broiler farms and nine feed dealers were visited. The surveys included interviews of the farmers and feed samplings. The interviews included questions on diet composition, the amount of diet, the amount of animal products (milk, meat and eggs), reproductive performance (litter size and breeding cycle), animal management (e.g., the animals' feeding period and body weights at the time of purchase and sale), and manure management. The production area of each feed was determined by

asking local animal farmers and feed dealers. A detailed description of the field survey was provided in the previous chapter.

K and Mg concentrations in feeds

The K and Mg concentrations in some of the feeds for cattle and pigs were obtained by chemical analyses (Table 3.1 and 3.2), and those of the other feeds were obtained from MOA (2004a,b).

Feed samples were ground and dried in a drying oven at 60°C, and the dry matter (DM) contents were measured. In addition, the feed samples were wet-digested with nitric and perchloric acids, and the K and Mg concentrations in the samples were determined with an atomic absorption spectrophotometer (AA-6600F; Shimadzu, Kyoto, Japan).

Table 3.1 The amount of diets, diet composition and nutrient concentrations in feed used in the calculation of K and Mg budgets for dairy cattle

	Feed intake (kg/day)	DM (%)	K (%DM)	Mg (%DM)	Production area
Fermented corn residue	9.0	22.9	0.26	0.81	Local ³
Corn grain	6.3	88.4	0.31	0.16	External ⁴
Soybean curd residue ¹	2.5	10.1	1.72	0.28	Local ³
Rice bran ¹	1.0	90.2	1.73	0.90	External ⁴
Wheat flour ¹	0.3	87.8	0.60	0.41	External ⁴
Corn stem silage	4.2	22.7	1.48	0.88	External ⁴
Rice straw	2.1	95.7	1.69	0.20	External ⁴
Broad bean stem	1.5	84.9	1.23	0.43	Local ³
Vegetable residue ²	46.5	7.8	2.50	0.67	Local ³
Total	73.3	23.0	1.17	0.46	–

¹Dry matter, K and Mg contents were cited from MOA (2004a,b).

²Vegetable residue was assumed to be evenly halved broccoli and celtuce residues (K: 2.44% DM; Mg: 0.68% DM, and K: 2.62% DM; Mg: 0.64% DM, respectively) on a fresh weight basis.

³Feed produced within the system.

⁴Feed imported from outside of the system.

Table 3.2 Diet composition and nutrient concentrations in feed used in the calculation of K and Mg budgets for fattening pigs and breeding sows

	Ratio (%) ¹	DM (%)	K (%DM)	Mg (%DM)	Production area
<i>Fattening pigs</i>					
Rice molasses	69.2	23.3	0.11	0.08	Local ⁴
Corn grain	23.1	88.4	0.31	0.16	External ⁵
Rice bran ²	7.7	87.0	1.73	0.90	External ⁵
Total	100.0	62.2	0.46	0.24	
<i>Breeding sows</i>					
Corn grain	60.0	88.4	0.31	0.16	External ⁵
Soybean meal ²	20.0	89.0	1.77	0.25	External ⁵
Wheat bran ²	10.0	87.0	1.19	0.47	External ⁵
Fish meal ²	6.0	90.0	0.94	0.16	External ⁵
Vitamin supplement ³	4.0	90.0	–	–	External ⁵
Total	100.0	88.5	0.72	0.20	–

¹Fresh weight ratio in the total feed.

²Dry matter, K and Mg concentrations were cited from MOA (2004a,b).

³Dry matter, K and Mg concentrations were cited from industrial data.

⁴Produced within the system.

⁵Imported from outside of the system.

Feed K and Mg composition and animal management

Dairy cattle

The K and Mg flows for the lifetime of a Holstein cow were evaluated. A heifer was grown for 2 years from 40 kg to 400 kg. After maturation, six calves (body weight (BW): 40 kg each) were delivered to the cow's age of 8 years (BW: 563 kg). It was assumed that five calves were sold immediately after their births, and one female calf was kept for

replacement to maintain the herd size. The annual milk yield and lactating period were set at 6,590 kg/year and 305 days/year, respectively. The diet composition, dry matter, K and Mg concentrations in each feed and feed origins and the intake in lactation periods are shown in Table 3.1.

The types of vegetable residues differed from day to day. Since broccoli and celtuce residues were fed most frequently in the area during the surveyed periods, “vegetable residues” were assumed to be evenly halved broccoli and celtuce residues at a fresh weight basis for evaluation. The feed intakes during the growing and non-lactating periods were estimated from the equations of DM requirement obtained from NARO (2006), and the feed compositions were considered to be the same as those during the lactating periods.

Fattening pigs and breeding sows

A pig was fattened from 20 kg at the age of 2 months of introduction to 160 kg at the age of 8 months of finishing in fattening pigs. Rice molasses produced as a by-product of sugar/starch production at the farms were fed to pigs. Although the rice was imported from outside of the area, we treated rice molasses as local feed in the present study. Corn grain and rice bran were also fed in fixed proportions. The total feed intakes based on the digestible energy (DE) requirement were estimated from NARO (2005). In addition, a breeding sow and its piglets were considered in breeding sows in the study. A sow was purchased at the weight of 130 kg and culled at 230 kg after the sixth time farrow. One breeding cycle was set as 143 days, with the pregnancy, suckling and return of estrus periods of 115, 21 and 7 days, respectively. The litter size was set at 12 head (1.4 kg/head), and the lactation during the suckling period was 7.5 L/day. The total feed intake was

estimated from NARO (2005). Piglets were grown for 60 days and fed starter feed along with suckling. The K and Mg intakes of the piglets were assumed to be equivalent to the K and Mg requirements obtained from MOA (2004a). All of the feeds for the sows and piglets were commercially processed and packaged, and were imported from outside the area. The diet composition, DM contents, and K and Mg concentrations in feeds for fattening pigs and breeding sows are shown in Table 3.2.

Broilers and laying hens

A broiler was grown from 25 g at hatching to 3 kg at the age of 56 days of finishing, and a laying hen was fed from 20 g at hatching to 2 kg at the age of 450 days. Egg production began at the age of 130 days, and the lifetime egg yield of a hen was assumed to be 220 eggs (61 g/egg). Only the feed mixture imported from outside the area was used for both types of chickens. The feed intake for each week was derived from NARO (2011). The K and Mg concentration in the feed were derived from Rutherford *et al.* (2012) in broilers (K: 1.03% and Mg: 0.21%), and from Keshavarz and Austic (2004) and Hossain and Bertechini (1998) in laying hens (K: 0.68% and Mg: 0.16%).

Calculation of the K and Mg budgets at the individual level

The K and Mg budgets for each animal category were calculated following the procedures as well as N and P described in the previous chapter. The amounts of K and Mg intakes, retained and produced as milk or egg, were estimated from the amounts and the K and Mg concentrations. The amounts of K and Mg excreted were calculated by subtracting the amount retained and produced as milk and egg from the intakes. Table 3.3

shows the K and Mg concentrations in the animal products used in the calculation of K and Mg budgets.

Table 3.3 Nutrient concentrations in animal products (g/kg) used in the calculation of K and Mg budgets

Item	K(g/kg)	Mg(mg/kg)
Cow milk	1.5 ¹	100 ¹
Egg (with shell)	1.3 ²	800 ²
Cattle body	1.8 ³	450 ³
Fattening pig body	2.0 ⁴	279 ⁵
Breeding sow body	1.7 ⁴	270 ⁵
Newborn piglet body	11.3 ⁴	324 ⁵
Piglet body at 20 kg	2.3 ⁶	320 ⁵
Broiler body	1.5 ⁶	333 ⁵
Young layer body	1.8 ⁶	300 ⁵
Laying hen body	2.0 ⁶	450 ⁵

¹From MEXT (2010).

²From McDonald et al. (1995).

³From NRC (2001).

⁴From De Boer *et al.* (1997).

⁵From Georgievskii *et al.* (1982).

⁶From De Boer *et al.* (2000)

Estimation of K and Mg flows in the animal production at a regional level

The framework of the nutrient flows at the regional level can be seen in the Figure 2.2 in the previous chapter. Only the flows relevant to animal production were assessed in the present study. The system boundary was set around the entire study area. The two types of feeds were distinguished based on their production areas; feeds produced within

the system were defined as ‘local feed’, and those imported from outside the system were defined as ‘external feed’. All nutrients contained in animal products (including retained nutrients in animals) were exported outside the system.

The K and Mg flows in the overall animal production system in the study area were predicted by multiplying the individual K and Mg budgets by the number of each animal in the area. Because beef cattle production was scarce in the area, ‘cattle’ classified in the statistical data could be assumed to be dairy cattle. Supposing that the supply-demand balance of piglets was maintained within the area, the proportions of sows, piglets and fattening pigs were predicted to be 4.7%, 23.8% and 71.4% of the total number of ‘pigs’, respectively. The numbers of broilers and laying hens were both estimated to be one-half of the number of ‘chickens’.

With respect to manure management, the animal excretion was collected without separation of urine and feces and then applied to cropland without sufficient composting. The animal farmers sold large amounts of unused manure to neighboring crop farmers or manure dealers when the animal farmers had no farmlands for feed crops. Therefore, manure produced by the local farms was assumed to be applied to crop fields within the area and recycled within the system. Potassium and Mg losses before the spreading of manure to the croplands were not considered.

3.3 Results and discussion

K and Mg concentrations in each feed

We compared the K and Mg concentrations in each feed (Tables 3.1 and 3.2) with published reports. Because there was no information for non-edible parts of broccoli and celtuce for feed in the literature, only the information on the edible-parts were compared. In addition, information on fermented corn residue and rice molasses has not been reported, and thus their K and Mg concentrations could not be compared with other reports. For K, the concentrations in feeds were similar to or lower than the published values, with the exception of broad bean stem: Broad bean stem: 0.75% DM, corn stem silage: 1.46% DM and rice straw: 1.64% DM given by NARO (2009). Corn grain: 0.29% DM by MOA (2004a,b). Broccoli: 3.27% DM by MEXT (2010). Celtuce: 6.00% DM by USDA (2014). The Mg concentrations in broccoli, celtuce, corn grain, corn stem silage and rice straw were higher than the published values, but the concentration in broad bean stem was lower than the published value: Broad bean stem: 0.64% DM, corn stem silage: 0.12% DM and rice straw: 0.13% DM given by NARO (2009). Corn grain: 0.11% DM by MOA (2004a,b). Broccoli: 0.24% DM by MEXT (2010). Celtuce: 0.51% DM by USDA (2014).

Lower K concentrations and higher Mg concentrations in broccoli and celtuce were thus obtained in the present study. This result conflicted with our expectation, which was that since the amount of K accumulated in soils in the present study area was several times higher than the annual application of K as fertilizer (Moritsuka *et al.* 2013), the plant K uptake would increase (Rice & Rice 2011). Moreover, as Mg and K ions mutually

interfere with each other (Adams *et al.* 2008), the Mg ion uptake was expected to be interfered with by the high presence of K ions in soil.

The unexpected results could be partly explained by other agricultural practices in the area. Sugiyama (1968) mentioned that the overapplication of N fertilizer interferes with K uptake by vegetables and Acquaaah (2009) reported that a K-deficiency symptom is intensified with the application of ammonia forms of N. Inden (1972) reported that continuous cropping in greenhouses tends to lead to the accumulation of Mg in soil, and excessive Mg can interfere with K uptake. Indeed, Wang *et al.* (2015) reported that the mean input of N from chemical fertilizer per crop in the area was approximately two times higher than those of the recommended standard established by the local government (28.8 vs. 13.1 g/m² per crop, spinach; and 75.6 vs. 27.9 g/m² per crop, celery); those authors noted that most farmers in the area cultivated as many as six or seven crops per year in greenhouses with year-around cultivation. Our present findings for broccoli and celtuce may be related to the overapplication of N fertilizer and/or to an excessive accumulation of Mg due to the very high crop intensity in the area. However, because the mechanisms underlying plant nutrient uptake are extremely complex, additional studies are needed to reveal the plant nutrient uptake in the area in detail.

K and Mg budgets at the individual level

Dairy cattle

The 'local feed' accounted for more than 50% of the total K and Mg intakes (Table 3.4), which was higher than that reported by Hristov *et al.* (2006, 2007) for Idaho dairy farms in the U.S. (K: 22% and Mg: 17%). Vegetable and flower residues represented 79%

of the K intake and 51% of the Mg intake via ‘local feed’, suggesting that these residues largely contributed to the local nutrient cycle.

The dietary K concentrations during the lactation period were lower than those reported previously (1.96% and 2.00% DM by Bannink *et al.* (1999) and Kume *et al.* (2004), respectively) despite the fact that the dietary K concentration of 1.17% DM (Table 3.1) was higher than the requirements published by NARO (2006) (0.80% DM) and by MOA (2004b) (0.9% DM). The average excreted K was approximately 50% lower than the previous values (Bannink *et al.* 1999; Kume *et al.* 2004), suggesting that K feeding in the area reduced K excretion with meeting the requirements, compared to the previous studies.

On the other hand, the dietary Mg concentration in the present study (0.46% DM, Table 3.1) was also higher than the lactation-period requirements established by NARO (2006) (0.20%–0.25% DM) and by MOA (2004b) (0.20% DM). In contrast to K, the Mg intakes during the lactation period were higher than the reported values (77 vs. 29.6–71.1 and 49 g/day by Holtenius *et al.* (2008) and Kamiya *et al.* (2010), respectively) and the average excreted Mg was also 21%–189% higher than the previous values (Holtenius *et al.* 2008; Kamiya *et al.* 2010). Accordingly, we inferred that the overfeeding of Mg might increase the excretion of Mg. The lower Mg use efficiency compared to that of K was related to the overfeeding of Mg (Table 3.4).

Table 3.4 Estimated K and Mg budgets for each animal category (kg/year/head)

	Dairy cattle		Fattening pigs		Breeding sows		Broilers		Laying hens	
	K	Mg	K	Mg	K	Mg	K	Mg	K	Mg
Feeder livestock	–	–	0.091	0.013	0.094	0.015	0.000	0.000	0.000	0.000
Total feed	55.187	21.697	4.079	2.193	8.971	1.793	0.457	0.093	0.249	0.059
Local feed ¹	32.180	13.194	0.094	0.276	–	–	–	–	–	–
External feed ²	23.007	8.504	3.985	1.917	8.971	1.793	0.457	0.093	0.249	0.059
Production	7.580	0.540	0.632	0.088	1.575	0.222	0.029	0.007	0.017	0.009
Excretion	47.601	21.160	3.538	2.117	7.489	1.586	0.428	0.087	0.231	0.049
Manure	47.601	21.160	3.538	2.117	7.489	1.586	0.428	0.087	0.231	0.049
Use efficiency (%) ³	13.7	2.5	13.3	3.4	16.5	11.6	6.37	6.94	6.98	16.12

¹Fermented corn residue, yellow-bean cake, broad-bean stem, and vegetable residue for dairy cows and rice molasses for fattening pigs.

²Feeds except local feed.

³ $(\text{Production} - \text{Feeder livestock}) / \text{Total feed} * 100$

Pig production

Most of the K and Mg were imported as ‘external feed’ in pig production. The dietary K and Mg concentrations were 0.46% and 0.24% DM in fattening pigs and 0.72% and 0.20% DM in breeding sows, respectively (Table 3.2). These values were higher than the requirements established by NARO (2005) (K: 0.20–0.35% DM and Mg: 0.046–0.080% DM) and by MOA (2004a) (K: 0.16–0.34% DM and Mg: 0.034–0.045% DM). Similar to the dairy cattle, the K intake (11.2 g/day) and excretion (9.7 g/day) in fattening pigs were lower than those reported by Mroz *et al.* (2002) (13.4 and 12.0 g/day for K intake and excretion), but the Mg intake (6.0 g/day) and excretion (5.8 g/day) in fattening pigs were higher than those provided by Adeola (1995) (1.4 and 1.0 g/day for Mg intake and excretion).

The differences of use efficiency between fattening pigs and breeding sows were larger in Mg than in K (Table 3.4). The larger difference of use efficiency might be related to the differences in the nutrient allocation in animal body, and production system. For the breeding sows, the entire bodies of their piglets reared with sows was expected to have higher Mg content than the fattened pigs, because 60% of the total Mg in the body is retained in bone and the proportion of bone weight per entire body weight is higher in younger animals. As a result, the conversion of feed Mg content into products would be higher in breeding sows than fattening pigs (12.4% vs. 4.0%).

Chicken production

Because only the typical purchased feed was used in the chicken production and the feed samples could not be obtained, the K and Mg concentrations were derived from the previous works (Rutherford *et al.* 2012; Keshavarz & Austic 2004; Hossain & Bertechini

1998). The estimated K use efficiencies of chickens were lower than other animal categories in the study (Table 3.4). The high Mg use efficiency in laying hens may be because of the eggs being rich in Mg (Table 3.4).

K and Mg flows in the animal production at the regional level

The estimated K and Mg flows in the animal production system in the area are shown in Figure 3.1. For the overall animal production, the K and Mg proportions of the 'local feed' to total feed were 31% and 37%, respectively. This result shows that the amounts of K and Mg imported from outside the area as 'external feed' exceeded the amounts of K and Mg that were exported outside the area as animal products, and the result shows that the amounts of K and Mg in the manure produced and applied in the area also exceeded the amounts of K and Mg used by animals as 'local feed'. These findings imply that the animal production in this region potentially increased the K and Mg loads in the regional agricultural system.

Of the feed nutrients consumed by the animals, 12% of the K and 4% of Mg were converted to livestock products, and 88% of the K and 96% of the Mg were recycled within the area by the application of manure. The total K and Mg amounts in manure were 338.66 and 143.32 t/year; those amounts divided by the area of agricultural land in the study site (2,799 ha) were 121 and 51 kg/ha/year, respectively.

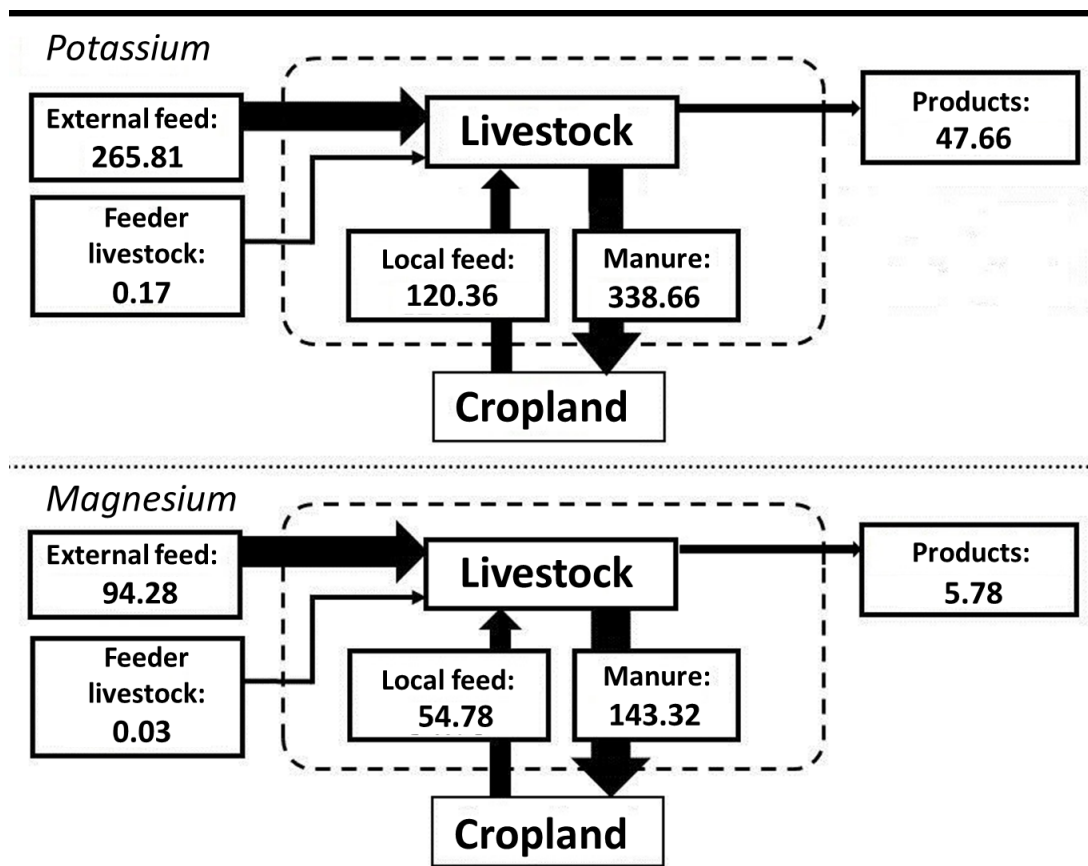


Figure 3.1 Potassium (upper) and magnesium (lower) flows in animal production at the regional level (t/year). Width of arrows indicates each nutrient amount.

Comparison of K and Mg flows with those of N and P

Individual level

Figure 3.2 shows a comparison of K and Mg local feed ratios and use efficiency (%) in animal production at the individual level and those of N and P reported in the previous chapter. The use efficiencies of K and Mg for all animal categories were lower than those of N and P. This might be because the transfer of nutrients from feed to products (milk, retention and egg) was lower in K and Mg than N and P. Moreover, in the Mg budgets of dairy cattle and pigs, the excessive Mg feeding compared to previous investigations was also related to the lower use efficiencies.

In dairy cattle, the local feed ratios of K and Mg were comparable to that of N and P. In contrast, the local feed ratios in the fattening pigs were lower in K and Mg than N and P because rice molasses (which is the only ‘local feed’ used for the fattening pigs) contained less K and Mg and thereby contributed less to the nutrient cycle within the area. This result for fattening pigs suggests that other ‘local feed’ should be used if enhancement of the local nutrient cycling of K and Mg is needed. In addition, as mentioned in the previous chapter, since all feed for breeding sows and chicken productions was ‘external feed’, nutrients could be utilized more efficiently by replacing ‘external feed’ with ‘local feed’.

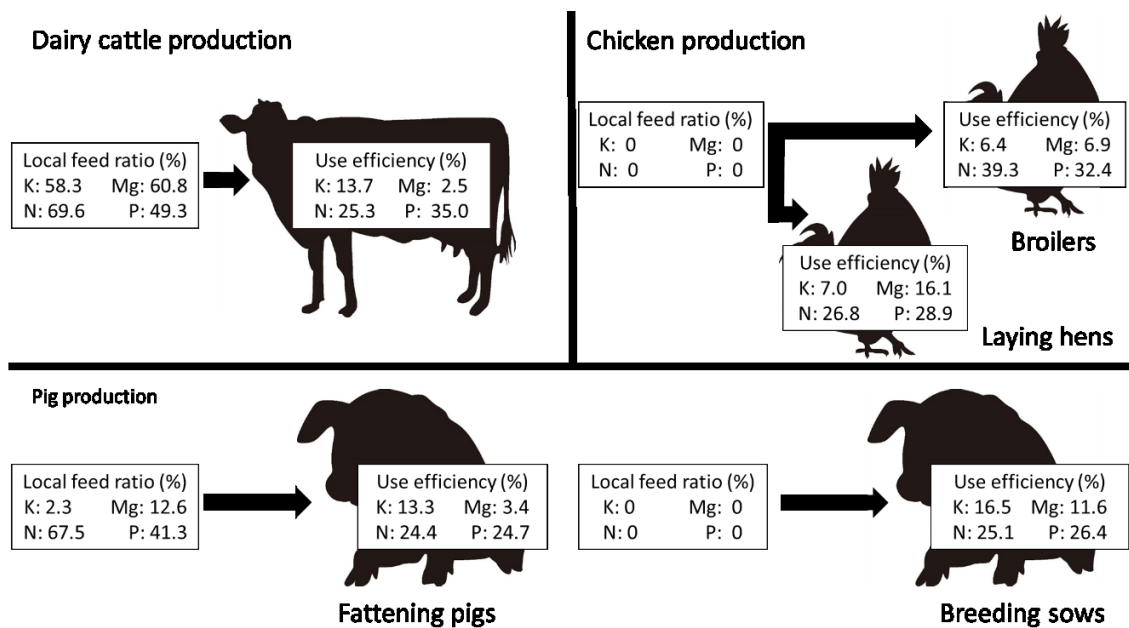


Figure 3.2 Estimated potassium, magnesium, nitrogen and phosphorus local feed ratio and use efficiency (%). Those of nitrogen and phosphorus were calculated in the previous chapter.

The K, Mg, N and P use efficiencies calculated for the nutrient flow in Idaho dairy farms in the U.S. reported by Hristov *et al.* (2006, 2007) were 9.2%, 5.1%, 20.6% and 27.4%, respectively, and those calculated in conventional dairy farms in Sweden reported by Gustafson *et al.* (2007) were 10.1%, 6.4%, 25.2% and 36.2%, respectively. Those of K, N and P in the present study's dairy farms were similar to or higher than those in the previous works. In contrast, the Mg use efficiency in the present study's dairy farms was lower than those reported previously (2.5% vs. 5.1% and 6.4% reported by Hristov *et al.* (2007) and Gustafson *et al.* (2007), respectively), which might be related to nutrient intake or concentration. The N intake at lactation periods was 17% higher than the requirement and the dietary P concentration at lactation periods was approximately equal to the requirement (described in the previous chapter), and that of K (1.17% DM) was approximately 40% higher than the requirements (0.80 or 0.90% DM), while that of Mg (0.46% DM) was as much as 130% higher than the requirements (0.20–0.25%). The high Mg intake due to the high dietary Mg concentration might have resulted in the low Mg use efficiency. We accordingly inferred that the total dietary Mg concentration should be decreased with increasing local feed ratio to prevent the accumulation of Mg in the study area.

With respect to pigs, laying hens and broilers, because there was no report on Mg flow, only the use efficiencies of K, N and P could be compared to those in previous works. Those of N and P in pig production of the present study were comparable with previous data, but those of K were higher than the previous data: K, 4.6%–8.8%, N, 21.1%–41.0% and P, 11.9%–23.7% reported by Hedlund *et al.* (2003) for breeding sows and fattening farms in Vietnam; K, 6.7%, N, 32.8% and P, 35.1% reported by De Boer *et al.* (1997) for pig production in the Netherlands in 1990. The higher K use efficiency could be also

explained by the nutrient intake or concentration. In the study area, the N intakes of pigs were equal to the requirement and the dietary P concentration in pigs was 18% lower than the requirement (described in the previous chapter). In contrast, the dietary K concentration (0.46% DM for fattening pigs and 0.72% DM for breeding sows) were higher than the requirements (0.16%–0.35% DM). However, the K content in most practical diets was normally adequate to meet the requirement, and the K intake of the fattening pigs in the present study area (11.2 g/day) was lower than that reported by Mroz *et al.* (2002) (13.4 g/day) which was due to the lower K content of rice molasses than that of typical feed ingredients such as soybean meal and wheat bran. The K intake in pig production of the present study area might thus be lower than that of a practical diet, which might be related to the high K use efficiency. The K use efficiency in chicken production in the present study was slightly lower than the data published by De Boer *et al.* (2000), in their study of nutrient flow for chicken production in the Netherlands in 1990 (K, 9.1%, N, 32.5% and P, 22.2% in laying hens; K, 7.1%, N, 39.6% and P, 37.5% in broilers).

Regional level

Table 3.5 shows a comparison of the flows of K and Mg in animal production at the regional level with those of N and P provided in the previous chapter. Among all animal categories, the amounts of K, Mg and N in manure from chicken were the lowest, but that of P in manure was the lowest for dairy cattle. The amounts of N and P in manure were 52% and 201% higher from pigs than from cattle, respectively. By contrast, the amounts of K and Mg in manure were 90% and 53% higher from cattle than from pigs, respectively. These results suggest that the characteristics of the nutrient load on the cropland from

manure differed depending on the animal category in the present study area, and they provide useful information to reduce the nutrient load on the cropland from animal production.

Dietary manipulation to reduce nutrient excreta from animals

Feasible approaches to reducing nutrient emissions from animal production are reducing the total amount of nutrients in the diet and improving the efficiency of animal nutrient use (Ferket *et al.* 2002; Hristov *et al.* 2007). It has been difficult, however, to improve the availability of nutrient use because it can be influenced by other nutrient contents, the forms of feed (organic or inorganic), ambient temperatures and the ages of the animals (NRC 2001). Thus the reduction of the dietary concentrations of nutrients can be a relatively easy and efficient way of reducing the overall excretions of nutrients. Dietary manipulations have been successful in reducing the excretion of environmentally important nutrients such as P (Valk *et al.* 2000; Cerosaletti *et al.* 2004; Hristov *et al.* 2006) and N (Shriver *et al.* 2003; Olmos Colmenero & Broderick 2006; Kerr *et al.* 2006). Kojima *et al.* (2005) also reported that a decrease of K intake by dry cows could decrease urinary N excretion as well as K excretion. Buff *et al.* (2005) found that there was a linear increase in the Zn excreted with increasing dietary Zn-polysaccharide (an organic source of Zn) in weanling pigs, suggesting a reduction of the amount of excreted Zn by dietary manipulation. Dietary manipulations can also be expected to be successful in reducing the amount of Mg in manure in the present study area.

Table 3.5 Estimated K, Mg, N and P flows in animal production at the regional level (t/year)

	Cattle				Pig				Chicken			
	K	Mg	N ¹	P ¹	K	Mg	N ¹	P ¹	K	Mg	N ¹	P ¹
Feeder livestock	–	–	–	–	0.1	0.0	2.2	0.3	0.0	0.0	0.6	0.1
Total feed	202.7	79.7	406.6	56.2	106.8	52.9	687.0	146.4	76.7	16.5	252.1	54.6
Local feed	118.2	48.5	283.0	27.7	2.2	6.3	400.4	52.8	–	–	–	–
External feed	84.5	31.2	123.5	28.5	104.6	46.6	286.6	93.7	76.7	16.5	252.1	54.6
Production	27.8	2.0	102.7	19.7	14.7	2.1	170.5	36.8	5.1	1.7	86.9	16.9
Excretion	174.8	77.7	303.9	36.5	92.2	50.9	518.7	109.9	71.6	14.8	165.7	37.8
Manure	174.8	77.7	273.5	36.5	92.2	50.9	415.0	109.9	71.6	14.8	116.0	37.8
NH ₃ volatilization	–	–	30.4	–	–	–	103.7	–	–	–	49.7	–

¹Provided from the previous chapter.

As described in the previous chapter, the P load by manure in the area was considerably high compared with EU regulations. Since the overfeeding of Mg was suggested in dairy cattle and pig production, dietary manipulation in these animals' production appeared to be an efficient way to reduce the amount of Mg in the total manure, and doing so could thereby mitigate the P impact from the manure application in the area (Nair *et al.* 2003; Josan *et al.* 2005).

3.4 Conclusion remarks

Our findings demonstrated that the animal production contributed to the K and Mg loads on the cropland in the study area. In the K and Mg budgets at the individual level, our results suggested that excessive Mg intake resulted in high Mg excretion and low use efficiency in dairy cattle and fattening pigs. Regarding the nutrient flows in the animal production on the regional level, the information on K and Mg flows in the present study associated with the N and P flows provided in the previous chapter will be useful to reduce these nutrient loads from animal production on the cropland in the Dianchi Lake basin area.

CHAPTER 4

Dietary nitrate loads on cows in dairy farms in the southeastern basin of Dianchi Lake

4.1 Introduction

There have been numerous reports of deaths in ruminant animals due to nitrate toxicity (Nielsen & James 1992). Nitrates (NO_3^-) ingested by ruminant animals are generally reduced to ammonias through nitrites (NO_2^-) and hydroxylamines in the rumen (Barnet & Reid 1968). 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 (Smith and Jones 1957). 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 (Hungerford, 1970). 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 Dianchi Lake, 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 (Pietro 2006), 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.

4.2 Materials and methods

Site description

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

The agricultural characteristics of the study area obtained from the investigation can be seen in Table 2.1 in the chapter 2. 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.

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 (Cataldo *et al.* 1975). 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 (Hashimoto 1958).

4.3 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 4.1. Fermented corn residue, corn grain, rice bran, wheat flour, soybean curd residue, concentrate mixture and wheat bran

were fed to dairy cows as concentrates. Broad bean stem, rice straw and corn stem 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 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.

Table 4.1 Amounts of feedstuffs and proportions of vegetable and flower wastes at 5 investigated farms

Feeding amount (kg/day/head)	Farm A		Farm B		Farm C		Farm D		Farm E	
	FW	DM	FW	DM	FW	DM	FW	DM	FW	DM
Concentrates										
Fermented corn residue	10.0	2.3	8.0	1.8	5.0	1.1				
Corn grain	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						
Wheat flour	0.5	0.4								
Soybean curd residue	5.0	0.5								
Concentrate mixture					3.0	2.7				
Wheat bran							1.5	1.4		
Roughages										
Broad bean stem	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		
Corn stem 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

FW: fresh weight, DM: dry matter weight.

The nitrate nitrogen content in each feed is shown in Table 4.2. 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 provided by Bradley *et al.* (1940) (<0.1%DM; Table 4.3). 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) 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). 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 (<0.2%DM) given by MAFF (1988). It is known that ruminants often fall into nitrate poisoning when they ingest soiling crops containing high levels of nitrates in a short period (Miyazaki *et al.* 1974). Therefore, it was suggested that occurrence of acute toxicosis for the dairy cows may depend on the feeding management, ingredients and intervals.

Table 4.2 Nitrate nitrogen contents in feedstuffs (%DM)

Items	n	Mean	SD
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.	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.	-
Flower wastes			
Carnation	3	0.260	0.1005

SD: Standard deviation, N.D.: Not detected.

Table 4.3 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.

Quoted from Bradley *et al.* (1940)

The ratios of methemoglobin to the total hemoglobin ranged from 1.6% to 3.2% (mean: 2.2%) in June 2010 and from 3.0% to 8.0% (mean: 5.0%) in September 2010. 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% (Steven 1972). This also holds true in dairy cows (Li *et al.* 1961). Therefore, in the present study, the dairy cows were assumed not to have fallen into acute nitrate poisoning.

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. 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 (Yoshida 1994). 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.

CHAPTER 5

General Discussion

In the previous chapters, several problems were presented for animal production systems in the southeastern basin of Dianchi Lake that were associated with intensive agricultural production. The findings provided in Chapters 2 and 3 indicated that animal production might contribute to imbalances in N, P, K and Mg budgets in croplands through the considerable amounts of manure applied to fields. The results described in Chapter 4 suggested that animal health in local dairy farms could be threatened due to high levels of dietary nitrate introduced through the large amounts of vegetable and flower residues produced in the area. However, for sound and sustainable nutrient cycling in the regional agricultural systems, it is essential to evaluate the comprehensive nutrient flows in integrated animal and crop production systems.

In addition to the nutrient flows in animal production in the study area calculated herein, information on nutrient properties and balances in other systems in the area have become available from research collaborators from other disciplines, such as crop science, soil science, horticulture and hydrology. Moritsuka *et al.* (2013) investigated soil physicochemical properties in the study area and reported that the average amounts of readily available N, P and K that had accumulated in greenhouse soil exceeded 5,000 kg/ha and were equal to or higher than the annual input of those nutrients as fertilizers. Inorganic N, of which NO_3^- accounted for 459 mg/kg, as well as water-soluble P in the greenhouse soils were considerably higher than those in the paddy soils, and the accumulation of water-soluble cations, including Mg^{2+} , in the surface layer of greenhouse soil was indicated by more than 100% of the average base saturation percentage. Following the analyses of surface water samples in rivers that flow into Dianchi Lake from the study area, Tanaka *et al.* (2013) noted that N (NO_3^- -N and Total N) pollution in the rivers was particularly high (>10 mg/L and >25 mg/L, respectively) during the rainy

season, while cumulative P loads were observed in the lower reaches of the rivers where the predominant land use was greenhouse production. Wang *et al.* (2015) described the annual nutrient balance in heavy multiple cropping systems in the same area and reported that 58%, 72% and 20% of N, P and K inputs, respectively, were not absorbed by crops in the vegetable field.

To advance the general discussion, we attempted here to evaluate the regional nutrient cycling, exports and losses between animal and crop production systems. The N, P and K flows in the agricultural production system were estimated from the information on animal production given in Chapters 2 and 3, as well as information on crop production provided by Wang *et al.* (2015). Because a Mg budget was not determined for crop production, Mg flow could not be estimated. Surpluses and three flow indices (cycling, exports and loss indices) for N, P and K were calculated from the estimated flows. The surpluses were calculated by subtracting nutrient exports as agricultural products from nutrient inflows to the total system. The nutrient surplus can be considered a potential nutrient load to the environment. The three flow indices were calculated from following equations according to Kimura and Hatano (2007):

$$\begin{aligned} \text{Total system throughflow} &= \text{nutrient inflow to the total system} + \text{internal cycling flow} \\ &= \text{internal cycling flow} + \text{nutrient export as agricultural products} \\ &\quad + \text{surplus in the total system} \end{aligned}$$

where internal cycling flow means the sum of nutrients exchanged between animal and crop production within the system as local feed and manure.

Cycling index (CI) = internal cycling flow / total system throughflow

Export index (EI) = export by agricultural products / total system throughflow

Loss index (LI) = surplus in the total system / total system throughflow

The CI, EI and LI indicate the cycle, export and loss portions of the total system throughflow, respectively. Because the total system throughflow is the sum of the internal cycling flow, nutrient exports by agricultural products and surplus in the total system, the sum of CI, EI, and LI is equal to one. In addition to the N, P and K surpluses and the three flow indices in the study area, the surpluses and indices for N flow in four villages in Guanxi province (Liang *et al.* 2005) and two counties in the Yangtze river basin (Liu *et al.* 2007) and for N, P and K flows in dairy and beef cattle farms in Japan (Kobayashi *et al.* 2010; Tabata *et al.* 2009) were calculated to serve as references at the regional and farm scales, respectively.

The estimated N, P and K flows in the agricultural production system are shown in Figure 5.1. The N, P and K inflows from outside the system to crop production primarily originated from chemical fertilizers, which accounted for 85%, 84% and 90% of the total inflow of N, P and K to the system, respectively. The N, P and K exports from the crop production system came primarily from harvested vegetables, which accounted for 77%, 78% and 98% of the N, P and K exports from the system, respectively. For the total agricultural production system, 36% of N, 30% of P and 80% of K inputs were converted to agricultural products, while the other portions (i.e. surpluses) were not used for production and were considered to be potential environment loads.

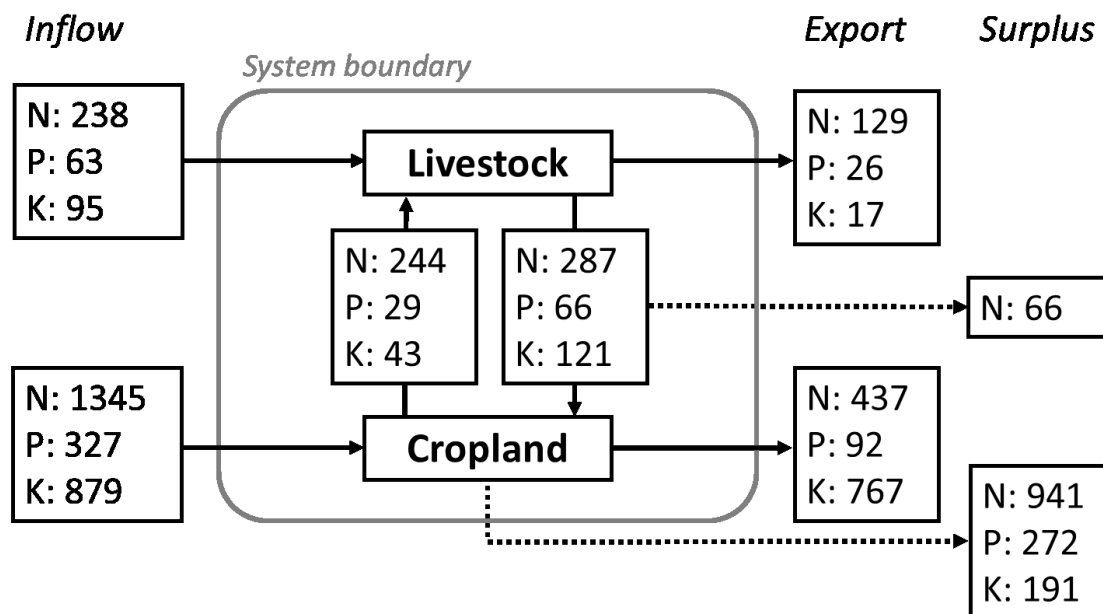


Figure 5.1 Nitrogen (N), phosphorus (P) and potassium (K) flows in the agricultural production system per area of agricultural land in the study area (kg/ha/yr).

The N, P and K surpluses and three flow indices are shown in Table 5.1. The N and P surplus in the study area was considerably higher than the reference values. These high N and P surpluses were mainly due to multiple cropping and an over application of chemical fertilizers, as repeatedly noted by Moritsuka *et al.* (2013) and Wang *et al.* (2015). However, the K surplus in the area was not higher than the values for Japanese dairy and beef cattle farms. This may be due to differences in the products exported from the systems. The major product in the study area was vegetables, whereas the products from the dairy and beef cattle farms were almost exclusively milk and beef. Because the K use efficiency for crop production, especially for some vegetables, was far higher than for animal production, the K inflow into the system was efficiently transferred to products despite the high inflow.

The CI for the N flow in the study area was lower than those calculated for the Japanese cattle farms, but it was equivalent to those calculated for the villages in Guanxi province and the two counties in the Yangtze River basin. The CIs for the P and K flows in the study area were lower than those for the Japanese cattle farms. Several by-products used as feeds, such as vegetable residues, largely contributed to the nutrient cycles, as suggested in Chapters 2 and 3. The relatively lower CIs in the study area were partly because feed crops were not typically produced within the area while the main products of the croplands were primarily produced for export. The EIs for the N, P and K flows in the study area were higher than the reference values. As suggested in Chapters 2 and 3, the use efficiencies of N, P and K in animal production (27%, 28% and 12%, respectively) were not lower than the values reported in previous studies. The relatively high EIs in the study area were considered to have resulted from the year-round and heavy multiple cropping systems to achieve high yields. The LI for the N flow in the study area was relatively lower than the reference values, while the LI for the P flow was slightly higher and for the K flow was lower than those for the Japanese cattle farms. Because the nutrient losses from the system were assumed to come only from cropland, except for ammonia volatilization from manure, the LIs for N, P and K were considered to approximately correspond to the ratios of N, P and K inputs not absorbed by the vegetable crops (58%, 72% and 20%, respectively) (Wang *et al.* 2015).

For sound and sustainable nutrient cycling in the study area, considerable reduction of nutrient surpluses will be required; subsequent decreases in the loss portion are also recommended. As Moritsuka *et al.* (2013) and Wang *et al.* (2015) repeatedly suggested, the nutrient inputs to greenhouse fields exceeded the requirement for the crops and consequently accumulated in the soils. Wang *et al.* (2015) also noted that the N absorption

ratios in the study area were lower than the values reported in Japan (Nishio *et al.* 2001) and could be improved by the use of slow-release fertilizers and split applications (Shaviv and Mikkelsen 1993; Sowers *et al.* 1994). Because the P input did not improve vegetable yields, a massive reduction in P application might be possible. Additionally, although K was relatively efficiently absorbed by crops, the K absorption ratios tended to decrease with successive years of greenhouse multiple cropping. Because the nutrient inflows to the system were greater for crop production than for animal production, reducing nutrient inputs to croplands and improving nutrient absorption ratios by crops are the most necessary and feasible ways to decrease surpluses. It might also be possible to decrease the loss portions of nutrient flows while keeping the present export portions because available nutrients have already accumulated in the soils (Moritsuka *et al.* 2013). Additionally, the high N inputs in crop production might result in high nitrate contents in some vegetables and flowers used as feed, especially for celery, as reported in Chapter 4. The mean input of N from chemical fertilizers to celery were 756 kg/ha/crop and were 2.7 times higher than the standard application amount to celery (279 kg/ha/crop) recommended by the local government (Wang *et al.* 2015). Consequently, the mean nitrate nitrogen content in celery in the study area (1.36% DM, 936 µg/g of fresh matter, see Chapter 4) was 1.6 times higher than the average value (583 µg/g of fresh matter) from 50 samples of celery cultivated in Japan (Fujinuma *et al.* 2007). Thus, appropriate levels of fertilization will be required to mitigate nitrate contents in crops.

Table 5.1 Surpluses and flow indices for N, P and K flows calculated from this thesis and references

Nutrient	Region	System boundary	Surplus (kg/ha/yr)	CI	EI	LI	Reference
N	Southeastern basin of Dianchi Lake	Region	1012	0.25	0.27	0.48	This thesis and Wang <i>et al.</i> (2015)
P			274	0.19	0.24	0.56	
K			192	0.14	0.69	0.17	
N	Nonshi-tun, Qibainong	Region (village scale)	348	0.19	0.10	0.71	Liang <i>et al.</i> (2005)
	Nongli-tun, Qibainong		606	0.17	0.08	0.75	
	Patan-tun, Qibainong		465	0.09	0.08	0.83	
	Waixian-tun, Qibainong		323	0.24	0.08	0.68	
N	Taoyuan county, Hunan province	Region (county scale)	186	0.26	0.20	0.54	Liu <i>et al.</i> (2007)
	Taihe county, Jiangxi province		92	0.33	0.26	0.41	
N	Tochigi prefecture	Farm (dairy)	378	0.32	0.17	0.51	Kobayashi <i>et al.</i> (2010)
P			97	0.35	0.13	0.53	
K			199	0.59	0.07	0.34	
N	Kyoto prefecture	Farm (beef cattle)	436	0.42	0.05	0.54	Tabata <i>et al.</i> (2009)
P			136	0.51	0.03	0.46	
K			263	0.70	0.00	0.30	

CI: cycling index, EI: export index, LI: loss index, defined by Kimura and Hatano (2007)

As suggested in Chapter 3, reducing the total amount of nutrients in the diet and improving the efficiency of animal nutrient use are one of the approaches to reducing nutrient emissions from animal production (Ferket *et al.* 2002; Hristov *et al.* 2007). Dietary manipulations have been successful in reducing the excretion of N (Shriver *et al.* 2003; Olmos Colmenero & Broderick 2006; Kerr *et al.* 2006) and P (Valk *et al.* 2000; Cerosaletti *et al.* 2004; Hristov *et al.* 2006) in previous studies. However, dietary manipulation will have smaller effects on N and P use efficiencies in the study area because the amounts of N and P fed to animals were approximately close to those necessary to meet the dietary requirement. Dietary manipulations may be successful in reducing the amount of K and Mg in manure in the study area because these nutrients far exceeded the dietary requirements of several animal categories. Increasing the utilization of local feed material in animal production can facilitate nutrient cycling within a system. Kimura and Hatano (2007) noted that in an agroecosystem, the goal is not to increase internal cycling flows but rather to increase production while minimizing losses. However, because the production system in the study area has been intensified, there is limited capacity for increasing production. Hence, an increase in internal cycling with constant production can indirectly contribute to decreases in the loss portion of nutrient flows. Because feeding large amounts of vegetables can increase the risk of nitrate poisoning, as suggested in Chapter 4, the development of other feed resources is preferable for facilitating nutrient cycling within the region. For instance, Tanaka *et al.* (2016) investigated the potential use of a common reed grown in Dianchi Lake as roughage for ruminants by analyzing its nutritive value. The authors recognized its possibility as a high-quality roughage (containing 15% of crude protein and 60% of total digestible nutrients) if shoots were harvested in the early growing stage. The use of this new feed

resource will be effective for increasing the regional nutrient cycle, decreasing nutrient inflows to the system as feed and removing extra nutrients from the lake. The comprehensive use of manure and local feed nutrients in the broader region will also be promote efficient nutrient cycling because the required feed nutrients and the applied manure nutrients per area of agricultural land tended to be high in association with the high livestock densities, as suggested in Chapter 2. In conclusion, these comprehensive approaches to appropriate nutrient management in both crop and animal production systems are required for sound and sustainable nutrient cycling in regional agricultural systems and are important steps for mitigating the water pollution and eutrophication of the aquatic systems in the Dianchi Lake basin.

REFERENCES

REFERENCES

- Aarts HFM, Habekotté B, Van Keulen H. 2000a. Nitrogen management in the “De Marke” dairy farming system. *Nutrient Cycling in Agroecosystems* 56, 231-240.
- Aarts HFM, Habekotté B, Van Keulen H. 2000b. Phosphorus management in the “De Marke” dairy farming system. *Nutrient Cycling in Agroecosystems* 56, 219-229.
- Acquaah G. 2009. *Horticulture: Principles and practices*, 4th edn. Pearson Education, New Jersey, USA.
- Adams CR, Bamford KM, Early MP. 2008. *Principles of horticulture*, 5th edn. Butterworth-Heinemann, Oxford, UK.
- Adeola O. 1995. Digestive utilization of minerals by weanling pigs fed copper- and phytase-supplemented diets. *Canadian Journal of Animal Science* 75, 603–610.
- Agriculture Research Council (ARC). 1980. *The Nutrient Requirements of Ruminant Livestock*. Commonwealth Agricultural Bureaux, London.
- Amachika Y, Anzai H, Wang L, Oishi K, Irbis C, Li KZ, Kumagai H, Inamura T, Hirooka H. 2014. Concentrations of cadmium and lead in milk and feed in dairy farms in a region located in Kunming City, Yunnan Province, China. *Trace Nutrient Research* 31, 45–50.
- AOAC. 2000. *Official Methods of Analysis*, 17th edn. Association of Official Analytical Chemists, Arlington, VA.
- Bannink A, Valk H, Van Vuuren AM. 1999. Intake and excretion of sodium, potassium, and nitrogen and the effects on urine production by lactating dairy cows. *Journal of Dairy Science* 82, 1008–1018.

- Barnett AJG, Reid RL. 1961. *Reactions in the Rumen*, pp.198-203. Edward Arnold, London.
- Barry DAJ, Goorahoo D, Goss MJ. 1993. Estimation of nitrate concentrations in groundwater using a whole farm nitrogen budget. *Journal of Environmental Quality* 22, 767-775.
- Bouwman L, Goldewijk KK, Van Der Hoek KW, Beusen AHW, Van Vuuren DP, Willems J, Rufino MC, Stehfest E. 2011. Exploring global changes in nitrogen and phosphorus cycles in agriculture induced by livestock production over the 1900–2050 period. *Proceedings of the National Academy of Sciences*. USA, Available from URL: <http://www.pnas.org/cgi/doi/10.1073/pnas.1012878108>
- Bradley WB, Eppson HF, Beath OA. 1940. Livestock poisoning by oat hay and other plants containing nitrate. *Wyoming Agricultural Experiment Station Bulletin*, 241.
- Buff CE, Bollinger DW, Eilersieck MR, Brommelsiek WA, Veum TL. 2005. Comparison of growth performance and zinc absorption, retention, and excretion in weanling pigs fed diets supplemented with zinc-polysaccharide or zinc oxide. *Journal of Animal Science* 83, 2380–2386.
- Cataldo DA, Haroon M, Schrader LE, Younds VL. 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Soil Science and Plant Analysis* 6, 71-80.
- Cerosaletti PE, Fox DG, Chase LE. 2004. Phosphorus reduction through precision feeding of dairy cattle. *Journal of Dairy Science* 87, 2314–2323.
- Chase LE. 2003. Nitrogen utilization in dairy cows—what are the limits of efficiency? In: *Proceedings of the Cornell Nutrition Conference*, pp. 233-244. Syracuse, New York.

- De Boer IJM, Peters HTA, Grossman M, Koops WJ. 1997. Nutrient flows in agriculture in The Netherlands with special emphasis on pig production. *Journal of Animal Science* 75, 2054–2063.
- De Boer IJM, Van Der Togt PL, Grossman M, Kwakkel RP. 2000. Nutrient flows for poultry production in The Netherlands. *Poultry Science* 79, 172–179.
- Dutch Ministry of Economic Affairs, Agriculture and Innovation. 2010. Fourth Action Programme Nitrate Directive (2010-2013). Available from URL:
http://www.hetInvloket.nl/portal/page?_pageid=116,1640360&_dad=portal&_schema=PORTAL&p_file_id=2000343
- European Commission. 2011. On implementation of Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources based on Member State reports for the period 2004-2007. Available from URL:
http://ec.europa.eu/environment/water/water-nitrates/pdf/sec_2011_909.pdf
- Fahmy MH. 1972. Comparative study of colostrum and milk composition of seven breeds of swine. *Canadian Journal of Animal Science* 52, 621-627.
- Fangueiro D, Pereira J, Coutinho J, Moreira N, Trindade H. 2008. NPK farm-gate nutrient balances in dairy farms from Northwest Portugal. *European Journal of Agronomy* 28, 625–634
- FAO. 2012. FAOSTAT. Available from URL: <http://faostat3.fao.org/home/index.html>
- Ferket PR, van Heugten E, van Kempen TATG, Angel R. 2002. Nutritional strategies to reduce environmental emissions from nonruminants. *Journal of Animal Science* 80, E168–E182.

- Finn JT. 1980. Flow analysis of models of the Hubbard Brook ecosystem. *Ecology* 61, 562–571.
- Fujinuma K, Ibe A, Tabata S, Hashimoto H, Saito K, Nakazato M, Ishikawa F, Moriyasu T, Shimamura Y, Kikuchi Y, Ogawa H, Ushiyama H, Yokoyama K, Yasuda K. 2007. Contents of Nitrate and Nitrite in Vegetables and Fruits. *Annual Report of Tokyo Metropolitan Institute of Public Health* 58: 195-203.
- Gao C, Zhu JG, Dou YJ. 2002. Contribution of agricultural non-point source pollution to water quality deterioration in Tai lake watershed: recent trends and research priorities. *Resources and Environment in the Yangtze Basin* 11, 260–263.
- Georgievskii VI, Annenkov BN, Samokhin VI. 1982. *Mineral Nutrition of animals*. Butterworths, London, UK.
- Gerber P, Chilonda P, Franceschini G, Menzi H. 2005. Geographical determinants and environmental implications of livestock production intensification in Asia. *Bioresource Technology* 96, 263-276.
- Gomori G. 1942. A modification of the colorimetric phosphorous determination for use with the photoelectric colorimeter. *Journal of Laboratory and Clinical Medicine* 27, 955-960.
- Goss MJ, Goorahoo D. 1995. Nitrate contamination of groundwater: measurement and prediction. *Fertilizer Research* 42, 331-338.
- Greenhouse Gas Inventory Office of Japan, Center for Global Environmental Research, National Institute for Environmental Studies. 2014. National Greenhouse Gas Inventory Report of JAPAN. National Institute for Environmental Studies, Tsukuba. Available from URL: <http://www-gio.nies.go.jp/aboutghg/nir/2014/NIR-JPN-2014-v3.0.pdf>

- Guo H, Li G, Zhang D, Zhang X, Lu C. 2006. Effects of water table and fertilization management on nitrogen loading to groundwater. *Agricultural Water Management* 82, 86-98.
- Gustafson GM, Salomon E, Jonsson S. 2007. Barn balance calculations of Ca, Cu, K, Mg, Mn, N, P, S and Zn in a conventional and organic dairy farm in Sweden. *Agriculture, Ecosystems and Environment* 119, 160–170.
- Halberg N., 1999. Indicators of resource use and environmental impact for use in a decision aid for Danish livestock farmers. *Agriculture, Ecosystems and Environment* 76, 17–30.
- Hashimoto T. 1958. Scrutiny of Evelyn and Malloy method of methemoglobin determination. *National Sanitation* 27, 385-387.
- Hatano R, Shinano T, Taigen Z, Okubo M, Zuowei L. 2002. Nitrogen budgets and environmental capacity in farm systems in a large-scale karst region, southern China. *Nutrient Cycling in Agroecosystems* 63, 139-149.
- Hayashi Y, Hatano R. 1999. Annual nitrogen leaching in subsurface-drained water from a clayey aquic soil growing onions in Hokkaido, Japan. *Soil Science and Plant Nutrition* 45, 451-459.
- Hechrath G, Brooks PC, Poulton PR, Goulding KWT. 1995. Phosphorus leaching from soils containing different phosphorus concentrations in Broadbald experiment. *Journal of Environmental Quality* 24, 904-910.
- Hedlund A, Witter E, An BX. 2003. Assessment of N, P and K management by nutrient balances and flows on peri-urban smallholder farms in southern Vietnam. *European Journal of Agronomy* 20, 71–87.

- Holtenius K, Kronqvist C, Briland E, Spörndly R. 2008. Magnesium absorption by lactating dairy cows on a grass silage-based diet supplied with different potassium and magnesium levels. *Journal of Dairy Science* 91, 743–748.
- Hossain SM, Bertechini AG. 1998. Effects of varying levels of magnesium and available phosphorus on performance of layers. *Animal Feed Science and Technology* 71, 363–368.
- Hristov AN, Hazen W, Ellsworth JW. 2006. Efficiency of use of imported nitrogen, phosphorus and potassium and potential for reducing phosphorus imports on Idaho dairy farms. *Journal of Dairy Science* 89, 3702–3712.
- Hristov AN, Hazen W, Ellsworth JW. 2007. Efficiency of use of imported magnesium, sulfur, copper, and zinc in Idaho dairy farms. *Journal of Dairy Science* 90, 3034–3043.
- Hungerford TG. 1970. *Diseases of Livestock*, pp.929-930. Angus and Robertson, Sydney.
- Inden T. 1972. *Environment and soil of glasshouse horticulture*. Shinbundo-shinkosha, Tokyo. (In Japanese)
- Japan Meteorological Agency. 2014. World Climate, ClimatView. Japan Meteorological Agency, Tokyo. Available from URL:
<http://ds.data.jma.go.jp/gmd/tcc/tcc/products/climate/climatview/frame.php>
- Jin XC. 2001. *Control and management technology of eutrophication lake*. Chemistry Industry Press, Beijing.
- Josan MS, Nair VD, Harris WG, Herrera D. 2005. Associate release of magnesium and phosphorus from active and abandoned dairy soils. *Journal of Environmental Quality* 34, 184–191.

- Kamiya Y, Kamiya M, Tanaka M. 2010. The effect of high ambient temperature on Ca, P and Mg balance and bone turnover in high-yielding dairy cows. *Animal Science Journal* 81, 482–486.
- Kerr BJ, Ziemer CJ, Trabue SL, Crouse JD, Parkin TB. 2006. Manure composition of swine as affected by dietary protein and cellulose concentrations. *Journal of Animal Science* 84, 1584–1592.
- Keshavarz K, Austic RE. 2004. The use of low-protein, low phosphorus, amino acid- and phytase-supplemented diets on laying hen performance and nitrogen and phosphorus excretion. *Poultry Science* 83, 75–83.
- Kimura SD, Hatano R. 2007. An eco-balance approach to the evaluation of historical changes in nitrogen loads at a regional scale. *Agricultural Systems* 94, 165-176.
- Kojima H, Kume S, Nonaka K, Oshita T, Kozakai T, Hirooka H. 2005. Effects of feeding and animal performance on nitrogen, phosphorus and potassium excretion by Holstein cows. *Animal Science Journal* 76, 139–145.
- Kume S, Nonaka K, Oshita T, Kozakai T, Kojima H. 2004. Potassium excretion of dry, pregnant and lactating cows fed forage. *Nihon Chikusan Gakkaiho* 75, 179–184. (In Japanese)
- Li CW, Garcia-Rivera J, Burris RH. 1961. Metabolism of nitrate by cattle. *Biochemical Journal* 81, 237-242.
- Li SJ. 2006. An approach to accelerating innovative development of the lake science. *Bulletin of the Chinese Academy of Sciences* 21, 399–405.
- Le C, Zha Y, Li Y, Sun D, Lu H, Yin B. 2010. Eutrophication of lake waters in China: cost, causes, and control. *Environmental Management* 45, 662-668.

- McDonald P, Edwards RA, Greenhalgh JFD. 1981. *Animal nutrition*. Longman, New York.
- Ministry of Agriculture, Forestry and Fisheries (MAFF). 1988. *Report on soil diagnostic criteria for feed crop fields in Kanto and Tokai regions*, Grassland experiment station. 20-25.
- Ministry of Agriculture of the People's Republic of China. 2004a. *Feeding Standard of Chicken*. China Agriculture Press, Beijing.
- Ministry of Agriculture of the People's Republic of China. 2004b. *Feeding Standard of Dairy Cattle*. China Agriculture press, Beijing.
- Ministry of Agriculture of the People's Republic of China. 2004c. *Feeding Standard of Swine*. China Agriculture press, Beijing.
- Ministry of Education, Culture, Sports, Science and Technology in Japan (MEXT). 2005. Standard Tables of Food Composition in Japan, Fifth Revised and Enlarged Edition. Available from URL:
http://www.mext.go.jp/b_menu/shingi/gijyutu/gijyutu3/toushin/05031802.htm
- Mishima S, Itahashi S, Kimura R, Inoue T. 2003. Trends of phosphate fertilizer demand and phosphate balance in farmland soils in Japan. *Soil Science and Plant Nutrition* 49, 39-45.
- Miyazaki A, Kawashima R, Uesaka S. 1974. Effect of repeated administration of nitrate on methemoglobin formation in sheep. *The Japanese Journal of Zootechnical Science* 45, 233-238.
- Moritsuka N, Nishikawa T, Yamamoto S, Matsui N, Inoue H, Li KZ, Inamura T. 2013. Changes in soil physicochemical properties following land use change from paddy

- fields to greenhouse and upland fields in the southeastern basin of Dianchi lake, Yunnan Province, China. *Pedosphere* 23, 169–176.
- Mosquera J, Monteny GJ, Erisman JW. 2005. Overview and assessment of techniques to measure ammonia emissions from animal houses: the case of the Netherlands. *Environmental Pollution* 135, 381–388.
- Mroz Z, Reese DE, Øverland M, van Diepen JTM, Kogut J. 2002. The effect of potassium diformate and its molecular constituents on the apparent ileal and fecal digestibility and retention of nutrients in growing-finishing pigs. *Journal of Animal Science* 80, 681–690.
- Nair VD, Graetz DA, Dooley DO. 2003. Phosphorus release characteristics of manure and manure-impacted soils. *Journal of Food, Agriculture and Environment* 1, 217–223.
- National Agriculture and Food Research Organization (NARO). 2005. *Japanese Feeding Standard for Swine*. Japan Livestock Industry Association, Tokyo.
- National Agriculture and Food Research Organization (NARO). 2006. *Japanese Feeding Standard for Dairy Cattle*. Japan Livestock Industry Association, Tokyo.
- National Agriculture and Food Research Organization (NARO). 2008. *Japanese Feeding Standard for Beef Cattle*. Japan Livestock Industry Association, Tokyo.
- National Agriculture and Food Research Organization (NARO). 2009. *Standard Tables of Feed Composition in Japan*. Japan Livestock Industry Association, Tokyo.
- National Agriculture and Food Research Organization (NARO). 2011. *Japanese Feeding Standard for Poultry*. Japan Livestock Industry Association, Tokyo.
- National Research Council (NRC). 1980. *Mineral Tolerance of Domestic Animals*. National Academy of Sciences, Washington, D.C., USA.

- National Research Council (NRC). 1994. *Nutrient Requirements of poultry* 9th revised edn. National Academy Press, Washington, D.C., USA.
- National Research Council (NRC). 2001. *Nutrient requirements of dairy cattle* 7th revised edn. National Academy Press, Washington, D.C., USA.
- National Research Council (NRC). 2012. *Nutrient Requirements of swine*. National Academy Press, Washington, D.C., USA.
- Nielsen DB, James LF. 1992. Economic impact of poisonous plants on livestock production. *Journal of Range Management* 45, 3-8.
- Nishio M. 2001. Analysis of the actual state of nitrogen application in arable farming in Japan. *Japanese Journal of Soil Science and Plant Nutrition* 72: 513-521.
- Öborn I, Edwards AC, Witter E, Oenema O, Ivarsson K, Withers PJA, Nilsson SI, Richert Stinzing A. 2003. Element balances as a tool for sustainable nutrient management: a critical appraisal of their merits and limitations within an agronomic and environmental context. *European Journal of Agronomy* 20, 211-225.
- OECD. 2001. *Environmental Indicators for Agriculture: Methods and Results. Vol. 3*. OECD, Paris, France.
- Oenema O. 2003. Approaches and uncertainties in nutrient budgets: implications for nutrient management and environmental policies. *European Journal of Agronomy* 20, 3-16.
- Oenema, O. 2004. Governmental policies and measures regulating nitrogen and phosphorus from animal manure in European agriculture. *Journal of Animal Science* 82, 196-206.

- Oishi K, Kumagai H, Hirooka H. 2011. Application of the modified feed formulation to optimize economic and environmental criteria in beef cattle fattening systems with food by-products. *Animal Feed Science and Technology* 16, 38–50.
- Olmos Colmenero JJ, Broderick GA. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *Journal of Dairy Science* 89, 1704–1712.
- Pietro S. 2006. Nitrate in vegetables: toxicity, content, intake and EC regulation. *Journal of the Science of Food and Agriculture* 86, 10–17.
- Qin BQ. 2002. Approaches to mechanisms and control of eutrophication of shallow lakes in the middle and lower reaches of the Yangze River. *Journal of Lake Science* 14, 193–202.
- Rice LW, Rice RP. 2011. *Practical horticulture* 7th edn. Prentice Hall, New Jearsy, USA.
- Rufino MC, Hengsdijk H, Verhagen A. 2009. Analysing integration and diversity in agroecosystems by using indicators of network analysis. *Nutrient Cycling in Agroecosystems* 84, 229–247.
- Rutherford SM, Chung TK, Thomas DV, Zou ML, Moughan PJ. 2012. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. *Poultry Science* 91, 1118–1127.
- Schröder JJ, Neeteson JJ. 2008. Nutrient management regulations in The Netherlands. *Geoderma* 144, 418-425.
- Shaviv A, Mikkelsen RL. 1993. Controlled-release fertilizers to increase efficiency of nutrient use and minimize environmental degradation - A review. *Fertilizer Research* 35: 1-12.

- Shriver JA, Carter SD, Sutton AL, Richert BT, Senne BW, Pettey LA. 2003. Effects of adding fiber sources to reduced-crude protein, amino acid-supplemented diets on nitrogen excretion, growth performance, and carcass traits of finishing pigs. *Journal of Animal Science* 81, 492–502.
- Smith HA, Jones TC. 1957. *Veterinary Pathology*, pp.575-576. Lea & Febigar, Philadelphia.
- Sowers KE, Pan WL, Miller BC, Smith JL. 1994. Nitrogen use efficiency of split nitrogen applications in soft white winter wheat. *Agronomy Journal* 86: 942-948.
- Statistics Bureau of Yunnan Province. 2000. *Yunnan Statistical Yearbook 2000*. China Statistical Press, Beijing.
- Statistics Bureau of Yunnan Province. 2011. *Yunnan Statistical Yearbook 2011*. China Statistical Press, Beijing.
- Steinshamn H, Thuen E, Bleken MA, Brenøe UT, Elerhult G, Yri C. 2004. Utilization of nitrogen (N) and phosphorus (P) in an organic dairy farming system in Norway. *Agriculture, Ecosystems & Environment* 104, 509-522.
- Steven CMD. 1982. Collective review: Methemoglobinemia. *Annals of Emergency Medicine* 11, 214-221.
- Sugiyama T. 1968. *Vegetable nutrition and physiology and management of fertilization*. Buneido-shuppan, Tokyo. (In Japanese)
- Tamminga S. 1992. Nutritional management of dairy cows as a contribution to pollution control. *Journal of Dairy Science* 75, 345–357.
- Tabata Y, Togo D, Kitagawa M, Oishi K, Kumagai H, Kume S, Hirooka H. 2009. Nitrogen, phosphorus and potassium utilization and their cycling in a beef-forage production system. *Animal Science Journal* 80, 475-485.

- Tanaka TS, Irbis C, Kumagai H, Inamura T. 2016. Timing of harvest of *Phragmites australis* (CAV.) Trin. ex Steudel affects subsequent canopy structure and nutritive value of roughage in subtropical highland. *Journal of environmental management* 166, 420-428.
- Tanaka T, Sato T, Watanabe K, Wang Y, Yang D, Inoue H, Li KZ, Inamura T. 2013. Irrigation system and land use effect on surface water quality in river, at lake Dianchi, Yunnan, China. *Journal of Environmental Sciences* 25, 1107–1116.
- Terada F, Kurihara M, Nishida T, Sioya S. 1997. Prediction of nitrogen excretion in lactating cows. *The Japanese Journal of Zootechnical Science* 68, 163-168.
- Tsuiki M, Harada Y. 1997. A computer program for estimating the amount of livestock wastes. *Journal of the Japanese Agricultural Systems Society* 13, 17-23.
- U.S. Department of Agriculture (USDA). 2014. National Nutrient Database for Standard Reference. USDA, Washington, D.C.; [cited 30 December 2014]. Available from URL: <http://www.ars.usda.gov/ba/bhnrc/ndl>
- Valk H, Metcalf JA, Withers PJA. 2000. Prospects for minimizing phosphorus excretion in ruminants by dietary manipulation. *Journal of Environmental Quality* 29, 28–36.
- Van Horn HH, Newton GL, Kunkle WE. 1996. Ruminant nutrition from an environmental perspective: factors affecting whole-farm nutrient balance. *Journal of Animal Science* 74, 3082–3102.
- Wan L, Hu QL, Lin X, Qiu ZL. 2007. Land use planning based on control of non-point source pollution in reservoir watersheds. *Water Resources Protection* 23, 71–74.
- Wang Y, Tanaka T, Inoue H, Li KZ, Yang D, Inamura T. 2015. Annual nutrient balance and soil chemical properties in heavy multiple cropping system in the coastal area

of southeast Lake Dianchi, Yunnan Province, China. *Plant Production Science* 18, 323–335.

Watanabe K. 1996. Egg and its processed product. In: Tasaki I (eds), *Encyclopedia of Animal Production*. pp. 461-475. Yokendo, Tokyo.

Yoshida N. 1994. Safety of roughages. In: *Guidebook of Roughage Quality Evaluation*, pp. 95-103. Japanese Grassland Society, Tokyo.

Zhang T, Zeng WH, Wang SR, Ni ZK. 2013. Temporal and spatial changes of water quality and management strategies of Dianchi Lake in southwest China. *Hydrol. Earth. Syst. Sci. Discuss.* 10, 15409e15432. Available from URL: <http://dx.doi.org/10.5194/hessd-10-15409-2013>