



## Effects of Feeding Oxalate Containing Grass on Intake and the Concentrations of Some Minerals and Parathyroid Hormone in Blood of Sheep

M. M. Rahman, T. Nakagawa<sup>1</sup>, M. Niimi, K. Fukuyama<sup>2</sup> and O. Kawamura\*

Faculty of Agriculture, University of Miyazaki, Japan

**ABSTRACT :** In order to determine whether oxalate from grasses affects feed intake, blood calcium (Ca) and other blood parameters of adult sheep, two feeding trials were conducted. In Trial 1, one group of sheep received guineagrass (0.47% soluble oxalate) and another group received setaria (1.34% soluble oxalate) for 28 d. In Trial 2, one group of sheep received guineagrass while another group received the same grass treated with an oxalic acid solution (at a rate of 30 g oxalic acid/kg dry matter of hay) for 72 d. All sheep received concentrate mixtures (0.5% of body weight) throughout the experiment. In both trials, it was observed that plasma Ca concentration (11.0-11.7 mg/dl) was significantly ( $p < 0.05$ ) lower in sheep fed high oxalate-containing grasses than in sheep fed low oxalate-containing grasses (12.4-13.7 mg/dl). No differences ( $p > 0.05$ ) were observed in concentrations of magnesium, phosphorus and parathyroid hormone in plasma between the feeding of low and high oxalate-containing grasses. In addition, no differences ( $p > 0.05$ ) were observed in roughage dry matter (DM) intake, total DM intake or body weight of sheep. This study suggests that sheep may consume oxalate-rich forage, but Ca bioavailability may decrease with increasing oxalate levels in the ration. (**Key Words :** Oxalate, Forage, Feed Intake, Calcium, Phosphorus, Parathyroid Hormone)

### INTRODUCTION

Oxalate is found at high concentrations in many forage plants, including buffelgrass (*Cenchrus ciliaris*), pangolagrass (*Digitaria decumbens*), setaria (*Setaria sphacelata*), napiergrass (*Pennisetum purpureum*) and kikuyugrass (*Pennisetum clandestinum*). For example, setaria, which is commonly consumed by ruminants, contains up to 5.6% soluble oxalate (Jones and Ford, 1972). Napiergrass represents a major feed source for ruminants in tropical and subtropical areas that also contains up to 3.8% soluble oxalate (Rahman et al., 2006; Rahman et al., 2010). Oxalate, consumed in sufficient quantity, can bind with calcium (Ca) in the intestines and the blood to form insoluble Ca oxalate, which may lead to low serum Ca

levels and renal failure (James, 1972). In the literature, most studies on ruminants have been conducted by artificially administering oxalic acid with the ground ration or in a capsule form (Duncan et al., 1997; Kyriazakis et al., 1998; Burritt and Provenza, 2000; Duncan et al., 2000) that can readily be taken by animals within a short period. However, very little attention has been focused on direct feeding of oxalate-rich or oxalate-treated forage grasses to ruminants. The objective of this study was to investigate feed intake and some blood parameters of sheep in response to feeding on grass hay that contained different concentrations of oxalate.

### MATERIALS AND METHODS

#### Animals and diets

Approval for care and use of animal used in this study was obtained from the Animal Care and Use Committee of the University of Miyazaki. Guineagrass (*Panicum maximum* cv. Natsukaze) and setaria (var. Splenda) were harvested at the early flowering stage for making hay. Hay was chopped (into roughly 2-3 cm segments) so that selection of particular plant parts by the animals during

\* Corresponding Author : Osamu Kawamura. Tel: +81(0)985-58-7148, Fax: +81(0)985-58-7259, E-mail: kawamura@cc.miyazaki-u.ac.jp

<sup>1</sup> Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, Japan.

<sup>2</sup> Sumiyoshi Livestock Science Station, University of Miyazaki, Japan.

Received December 9, 2010; Accepted February 21, 2011

feeding was minimized. Five Suffolk and four crossbred (Corriedale×Suffolk) adult sheep were used in this experiment, and two trials were conducted. Prior to beginning the treatment in each trial, all sheep were fed chopped guineagrass for approximately 14 d. In Trial 1, sheep in Group 1 (2 female Suffolk and 1 female crossbred) received guineagrass, and sheep in Group 2 (3 female Suffolk and 1 castrated crossbred) received setaria for 28 d. In Trial 2, sheep in Group 1 (2 female Suffolk, 1 female crossbred and 1 castrated crossbred) received guineagrass, and sheep in Group 2 (1 female Suffolk and 2 female crossbred) received the same grass treated with oxalic acid solution (at a rate of 30 g oxalic acid/kg dry matter of hay) for 72 d.

Five sheep (3 female Suffolk, 1 female crossbred and 1 castrated crossbred) were used in both trials. In Trial 2, two female Suffolk sheep used in the Trial 1 were not used and replaced by another two female crossbred sheep. For making treated hay, oxalic acid (reagent grade, Nacalai Tesque Inc., Kyoto, Japan) was diluted with water (1:10 w/v) and sprayed over chopped hay, followed by proper mixing. The treated hay was then sun-dried. Each sheep was housed in an individual pen with free access to water. Sheep were fed a ration composed of hay (*ad libitum*) and concentrate mixtures (0.5% of body weight), offered in the morning and evening. The ingredients and chemical composition of concentrate mixtures are presented in Table 1.

### Sampling

Body weight (BW) was recorded prior to the beginning of the experiment and at 2-week intervals. Daily records were kept on feed consumption. Representative samples of offered hay and orts were taken weekly for chemical analysis and dry matter (DM) determination. Blood samples (10 mL) were taken at approximate 7-d intervals from the jugular vein using heparinized collection tubes at 9:00 am (before feeding) and centrifuged at 3,000 rpm for 15 min

1 h of collection. Collected plasma was stored at -20°C until assayed.

### Chemical analysis

Concentrations of Ca and magnesium (Mg) in feed were determined (absorbance at 422.86 and 285.13 nm for Ca and Mg, respectively) by flame atomic absorption spectrophotometer, and phosphorus (P) was determined (absorbance at 410 nm) using the molybdenum blue method by ultraviolet-visible spectrophotometer (UV Mini 1240, Shimadzu, Japan) after wet digestion with nitric acid and hydrogen peroxide (Laboratory of Agricultural Chemistry, the University of Tokyo, 1978). Oxalate was measured following the method of Rahman et al. (2007).

Plasma samples were diluted 1:1 with an aqueous solution of 5% lanthanum chloride, then diluted 50-fold with water. Subsequently, concentrations of Ca and Mg in plasma were determined by flame atomic absorption spectrophotometer, following the method of Thomas and Skujins (1999). For P determination, plasma samples were deproteinized with acetic acid (0.05 M), following the method of Hunter (1957). The deproteinized samples were prepared using the molybdenum blue method, and color intensity was measured by ultraviolet-visible spectrophotometer. Parathyroid hormone (PTH) concentration in plasma samples was determined enzymatically using the hPTH-EASIA Kit (GenWay Biotech Inc., San Diego, CA).

Differences between the two ration treatments in each trial were tested by Student's t-test (Steel and Torrie, 1984) using a repeated measure (weekly) to investigate the feed intake and the concentrations of some minerals and PTH in blood of sheep in these trials.

## RESULTS

### Mineral and oxalate concentrations of grass hay

The mean values of mineral element and oxalate concentrations of grass hay in this study are shown in Table 2. The oxalate concentration of setaria (1.34%) was much higher than the guineagrass (0.47%). The Ca concentration of setaria (0.50%) was slightly higher than in the guineagrass (0.43%). The Mg (0.40%) and P (0.21%) concentrations of setaria were similar to the Mg (0.44%) and P (0.22%) concentrations of guineagrass. The ratios of

**Table 1.** Chemical composition of concentrate mixtures\*

Composition	Percentage of dry matter basis
Crude protein	14.0
Ether extract	2.5
Crude fiber	10.0
Ash	10.0
Calcium	0.8
Phosphorus	0.5
Soluble oxalate	Trace
Total digestible nutrients	69.0

\* Concentrate mixtures includes 47% grains (maize, barley and wheat), 33% brans (rice bran, barley bran, shochu distiller's by-product), 15% oil cakes (soybean oil cake, rapeseed oil cake) and 5% others (calcium carbonate, molasses, seaweed meal, salt, alfalfa meal).

**Table 2.** Chemical composition (% , dry matter basis) of grass hay

Composition	Hay	
	Guineagrass hay	Setaria hay
Soluble oxalate	0.47	1.34
Calcium	0.43	0.50
Magnesium	0.44	0.40
Phosphorus	0.22	0.21

**Table 3.** Intake of rations as affected by feeding of different concentrations of oxalate-containing hay (Trial 1)

Parameter	Guineagrass hay (Mean±SE)	Setaria hay (Mean±SE)	Significance
Initial body weight (kg)	58.1±5.69	49.5±6.65	NS
Final body weight (kg)	58.2±4.31	50.2±7.60	NS
Roughage dry matter intake (g/d)	489.0±42.15	521.0±47.75	NS
Roughage dry matter intake (g/kg W <sup>0.75</sup> )	177.0±13.89	193.0±14.80	NS
Total dry matter intake (g/d)	762.0±50.81	757.0±61.75	NS
Total dry matter intake (g/kg W <sup>0.75</sup> )	275.0±16.11	281.0±18.70	NS

SE = Standard error; NS = Not significant ( $p>0.05$ ).

**Table 4.** Some mineral and parathyroid hormone concentrations in blood plasma as affected by feeding of different concentrations of oxalate-containing hay (Trial 1)

Parameter	Guineagrass hay (Mean±SE)	Setaria hay (Mean±SE)	Significance
Calcium (mg/dl)	13.7±0.46	11.7±0.74	*
Magnesium (mg/dl)	1.1±0.09	1.2±0.09	NS
Phosphorus (mg/dl)	5.1±0.59	5.7±0.39	NS
Parathyroid hormone (pg/dl)	28.7±3.74	29.3±5.75	NS

SE = Standard error; NS = Not significant ( $p>0.05$ ); \*  $p<0.05$ .

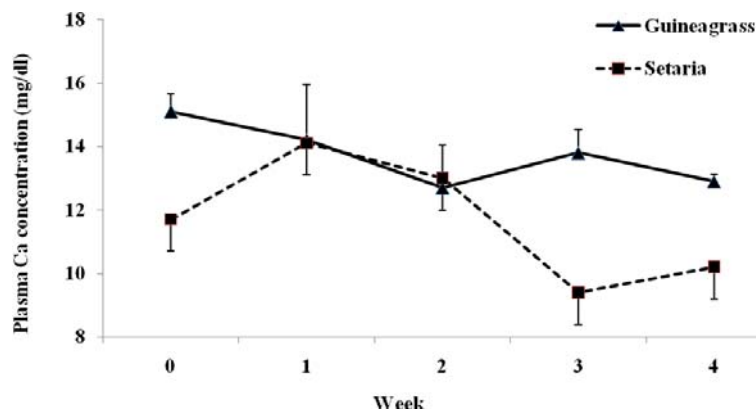
Ca and P in both grasses were almost 2:1, which is considered an ideal ratio for their relative concentrations in the diet, as reported by Whitehead (2000).

### Trial 1

The feeding effects of different concentrations of oxalate-containing grasses on BW and intake of rations are shown in Table 3. Feeding of low or high oxalate-containing grasses did not affect ( $p>0.05$ ) BW during the 28 d experimental period, which indicated that the experiment was carried out under normal conditions of animal health. No differences were observed ( $p>0.05$ ) between the feeding of guineagrass or setaria in roughage DM intake (489 compared with 521 g/d), roughage DM intake per metabolic weight (177 compared with 193 g/kg W<sup>0.75</sup>), total DM intake (762 compared with 757 g/d) or total DM intake per

metabolic weight (275 compared with 281 g/kg W<sup>0.75</sup>), respectively.

Table 4 shows the mineral and PTH concentrations in plasma of sheep fed guineagrass or setaria. The weekly plasma Ca concentrations of sheep were shown in Figure 1. Sheep fed setaria had lower ( $p<0.05$ ) Ca concentrations (11.7 mg/dl) than sheep fed guineagrass (13.7 mg/dl), even though the setaria contained more Ca than the guineagrass. There was no difference ( $p>0.05$ ) in Mg concentration in the plasma of sheep between the feeding of guineagrass (1.1 mg/dl) or setaria (1.2 mg/dl). Similarly, there was no difference ( $p>0.05$ ) in P concentration in the plasma of sheep between the feeding of guineagrass (5.1 mg/dl) or setaria (5.7 mg/dl). The PTH concentration in the plasma of sheep fed guineagrass (28.7 pg/dl) was similar ( $p>0.05$ ) to the PTH concentration of sheep fed setaria (29.3 pg/dl).

**Figure 1.** Plasma Ca concentration (mg/dl) of sheep (mean±standard error) fed the guineagrass hay or setaria hay (Trial 1).

**Table 5.** Intake of rations as affected by feeding of untreated and treated hay (Trial 2)

Parameter	Untreated hay (Mean±SE)	Treated hay <sup>1</sup> (Mean±SE)	Significance
Initial body weight (kg)	59.1±5.55	62.8±7.22	NS
Final body weight (kg)	62.6±5.15	63.6±8.54	NS
Roughage dry matter intake (g/d)	687.0±39.50	593.0±37.53	NS
Roughage dry matter intake (g/kg W <sup>0.75</sup> )	244.0±12.95	209.0±12.05	NS
Total dry matter intake (g/d)	990.0±46.33	911.0±46.00	NS
Total dry matter intake (g/kg W <sup>0.75</sup> )	353.0±14.87	322.0±14.13	NS

SE = Standard error; NS = Not significant ( $p>0.05$ ).

<sup>1</sup> Guineagrass hay was treated with oxalic acid at a rate of 30 g/kg dry matter of hay.

**Table 6.** Some mineral and parathyroid hormone concentrations in blood plasma as affected by feeding of untreated and treated hay (Trial 2)

Parameter	Untreated hay (Mean±SE)	Treated hay <sup>1</sup> (Mean±SE)	Significance
Calcium (mg/dl)	12.4±0.25	11.0±0.54	*
Magnesium (mg/dl)	1.1±0.08	1.1±0.10	NS
Phosphorus (mg/dl)	5.9±0.35	5.1±0.33	NS
Parathyroid hormone (pg/dl)	22.6±2.02	18.4±1.94	NS

SE = Standard error; NS = Not significant ( $p>0.05$ ); \*  $p<0.05$ .

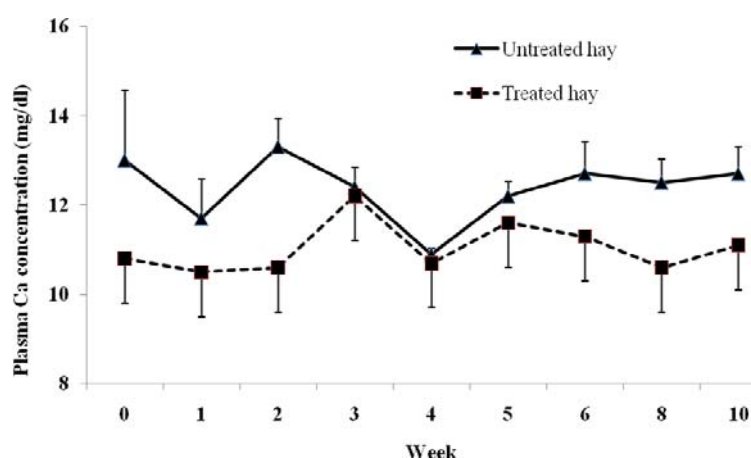
<sup>1</sup> Guineagrass hay was treated with oxalic acid at a rate of 30 g/kg dry matter of hay.

## Trial 2

The feeding effects of untreated and treated guineagrass on BW and intake of rations are shown in Table 5. Feeding guineagrass treated with or without oxalic acid had no effect ( $p>0.05$ ) on BW during the 72 d experimental period, which indicated that the experiment was carried out under normal conditions of animal health. There was no difference ( $p>0.05$ ) in roughage DM intake (687 compared with 593 g/d), roughage DM intake per metabolic weight (244 compared with 209 g/kg W<sup>0.75</sup>), total DM intake (990 compared with 911 g/d) or total DM intake per metabolic weight (353 compared with 322 g/kg W<sup>0.75</sup>) between the

feeding of untreated or treated guineagrass, respectively.

Table 6 shows the mineral and PTH concentrations in the plasma of sheep fed either untreated or treated guineagrass. The weekly plasma Ca concentrations of sheep were shown in Figure 2. Sheep fed untreated guineagrass had higher ( $p<0.05$ ) Ca concentrations (12.4 mg/dl) than sheep fed treated guineagrass (11.0 mg/dl). There was no difference ( $p>0.05$ ) in Mg concentration in the plasma of sheep between the feeding of untreated (1.1 mg/dl) or treated (1.1 mg/dl) guineagrass. Similarly, there was no difference ( $p>0.05$ ) in P concentration in the plasma of sheep between the feeding of untreated (5.9 mg/dl) or



**Figure 2.** Plasma Ca concentration (mg/dl) of sheep (mean±standard error) fed the untreated hay or treated hay<sup>1</sup> (Trial 2). <sup>1</sup> Guineagrass hay was treated with oxalic acid at a rate of 30 g/kg dry matter of hay.

treated (5.1 mg/dl) guineagrass. The PTH concentration in the plasma of sheep fed untreated guineagrass (22.6 pg/dl) was similar ( $p>0.05$ ) to the PTH concentration of sheep fed treated guineagrass (18.4 pg/dl).

## DISCUSSION

Some reports indicate that animals eat less food if it contains toxin (Burritt and Provenza, 2000), perhaps because of low palatability. Sheep in this study were offered either grasses with low concentrations of oxalate (guineagrass) or high concentrations of oxalate (setaria or treated guineagrass). Although feed intake appeared to decrease as the amount of oxalate in the ration increased (Trial 2), the difference was not statistically significant ( $p<0.05$ ). This result supports the findings of Wang and Provenza (1997) who reported that the presence of a toxin does not necessarily prevent ruminants from eating a food, especially if the food contains needed nutrients. In this study, the oxalate concentration of setaria was much less than the concentration reported by Jones and Ford (1972). This could have been due to the presence of some proportion of weeds in the field (data not shown), resulting in a lower oxalate concentration in setaria (Table 2).

Oxalic acid binds with Ca to form Ca oxalate, a non-soluble, non-digestible compound (Blaney et al., 1982). A mild degree of hypocalcaemia in sheep was observed at a dose of 0.12 g oxalic acid/kg BW/d (Kyriazakis et al., 1997), or at a dose of 0.24 g oxalic acid/kg BW/d (Kyriazakis et al., 1998). James and Butcher (1972) also reported that blood Ca decreased as the level of oxalate in the diet increased. The present study showed that sheep fed high oxalate-containing grass (0.13 and 0.34 g soluble oxalate/kg BW/d for Trial 1 and Trial 2, respectively) had lower plasma Ca concentration than sheep fed low oxalate-containing grass (0.04 and 0.05 g soluble oxalate/kg BW/d for Trial 1 and Trial 2, respectively). There was not a statistically ( $p>0.05$ ) significant difference in plasma Ca concentration between the groups when both the trials were initiated. This suggests that Ca bioavailability may decrease with increasing concentrations of oxalate in the ration. No toxicological symptoms were observed in the sheep, probably because plasma Ca concentrations of all sheep in all treatments (11.0-13.7 mg/dl) were in the normal range of Ca (9.0-12.0 mg/dl) as described by Kincaid (1993). This normal range of blood Ca may be the result of the concentrate supplement.

No changes in blood Mg concentration occurred due to feeding of oxalate-containing grasses, which is in agreement with the observations of James and Butcher (1972). James and Butcher (1972) also observed that no changes occurred in blood P concentration between the feeding of 0% or 4% oxalate-containing rations, but P

concentration increased with further increase (5-6%) in oxalate concentration in the ration. In the present study, grass hay contained up to 3.5% soluble oxalate, and no difference was observed in blood P concentration between the feeding of each type of ration in each trial.

Parathyroid hormone is secreted in response to low blood Ca concentrations, causing bone to release Ca into the blood stream. Low levels of the hormone are secreted even when blood Ca concentrations are high, but there is a steep increase in secretion of PTH when Ca concentrations fall below the normal range (Martin et al., 1996). In the present study, PTH concentrations in blood were not affected by feeding of oxalate-containing grasses in accordance with the normal range of plasma Ca concentrations observed in this study and described above.

If ruminants are gradually introduced to a high oxalate-containing ration over an approximately 3-4 day period, rumen microbes increase sufficiently to prevent oxalate poisoning (Allison et al., 1977). However, James et al. (1967) suggested that when sheep consume excessive amounts of an oxalate-containing plant, there is a depression of oxalate degradation in the rumen. James et al. (1967) observed that higher levels of oxalate (above 120 mg/100 mL rumen fluid) had a depressing effect on the efficiency of oxalate degradation. In this study, sheep were adapted to the guineagrass (0.47% soluble oxalate) for 2 weeks before starting the both experiments. Even though all sheep were adapted to the guineagrass, sheep fed high oxalate-containing grasses showed lower blood Ca concentrations than sheep fed low oxalate-containing grasses. However, sheep fed the high oxalate-containing grass for 72 d had nearly the same plasma Ca concentrations as the sheep fed the same grass for 28 d, suggesting that oxalate may be diluted across days due to increased ruminal metabolism (even after a period of adaptation). Further study is needed to determine how much oxalate could be included in feeds without reducing the availability of Ca.

In conclusion, feeding of high oxalate-containing grasses resulted in decreases in plasma Ca concentration. However, feed intake and concentrations of Mg, P and PTH in plasma of sheep were not affected by the feeding of low and high oxalate-containing grasses. This study suggests that sheep may consume oxalate-containing forage and may not show symptoms of toxicity even after feeding on high oxalate-containing forage, but Ca bioavailability may decrease with increasing oxalate levels in the ration.

## ACKNOWLEDGMENT

This work was supported by a Grant-in-Aid for Scientific Research from the JSPS (Japan Society for the

Promotion of Science). The award of a JSPS postdoctoral fellowship (No. P 09121) to M. M. Rahman is gratefully acknowledged.

## REFERENCES

- Allison, M. J., E. T. Littledike and L. F. James. 1977. Changes in ruminal oxalate degradation rates associated with adaptation of oxalate ingestion. *J. Anim. Sci.* 45:1173-1179.
- Blaney, B. J., R. J. W. Gartner and T. A. Head. 1982. The effects of oxalate in tropical grasses on calcium, phosphorus and magnesium availability to cattle. *J. Agric. Sci. Camb.* 99:533-539.
- Burritt, E. A. and F. D. Provenza. 2000. Role of toxins in intake of varied diets by sheep. *J. Chem. Ecol.* 26:1991-2005.
- Duncan, A. J., P. Frutos and S. A. Young. 1997. Rates of oxalic acid degradation in the rumen of sheep and goats in response to different levels of oxalic acid administration. *Anim. Sci.* 65:451-456.
- Duncan, A. J., P. Frutos and S. A. Young. 2000. The effect of rumen adaptation to oxalic acid on selection of oxalic-acid-rich plants by goats. *Br. J. Nutr.* 83:59-65.
- Hunter, G. 1957. A method for deproteinization of blood and other body fluids. *J. Clin. Pathol.* 10:161-164.
- James, L. F. 1972. Oxalate toxicosis. *Clin. Toxicol.* 5:231-243.
- James, L. F. and J. E. Butcher. 1972. Halogeton poisoning of sheep: Effect of high level oxalate intake. *J. Anim. Sci.* 35:1233-1238.
- James, L. F., J. C. S. Street and J. E. Butcher. 1967. *In vitro* degradation of oxalate and of cellulose by rumen ingesta from sheep fed *Halogeton glomeratus*. *J. Anim. Sci.* 26:1438-1444.
- Jones, R. J. and C. W. Ford. 1972. Some factors affecting the oxalate content of the tropical grass *Setaria sphacelata*. *Aust. J. Exp. Agric. Anim. Husband.* 12:400-406.
- Kincaid, R. 1993. Macro elements for ruminants. In: D. C. Church (eds) *The Ruminant Animal: Digestive Physiology and Nutrition*, pp. 326-341. Prentice Hall, Englewood Cliffs, NJ.
- Kyriazakis, I., T. G. Papachristou, A. J. Duncan and I. J. Gordon. 1997. Mild conditioned food aversions developed by sheep towards flavors associated with plant secondary compounds. *J. Chem. Ecol.* 23:727-746.
- Kyriazakis, I., D. H. Anderson and A. J. Duncan. 1998. Conditioned flavor aversions in sheep: the relationship between the dose rate of a secondary plant compound and the acquisition and persistence of aversions. *Br. J. Nutr.* 79:55-62.
- Laboratory of Agricultural Chemistry, the University of Tokyo (1978) *Jikken Nougei Kagaku*. Vol 1, pp. 276-277. Asakura Publishing Co. Ltd., Tokyo.
- Martin, K. L., R. M. Hoffman, D. S. Kronfeld and L. D. Warnick. 1996. Calcium decreases and parathyroid hormone increases in serum of periparturient mares. *J. Anim. Sci.* 74:834-839.
- Rahman, M. M., M. Niimi, Y. Ishii and O. Kawamura. 2006. Effects of season, variety and botanical fractions on oxalate content of napiergrass (*Pennisetum purpureum* Schumacher). *Grassl. Sci.* 52:161-166.
- Rahman, M. M., M. Niimi and O. Kawamura. 2007. Simple method for determination of oxalic acid in forages using high-performance liquid chromatography. *Grassl. Sci.* 53:201-204.
- Rahman, M. M., Y. Ishii, M. Niimi and O. Kawamura. 2010. Interactive effects of nitrogen and potassium fertilization on oxalate content in napiergrass (*Pennisetum purpureum*). *Asian-Aust. J. Anim. Sci.* 23:719-723.
- Steel, R. G. D. and J. H. Torrie. 1984. *Principles and Procedures of Statistics: A Biometrical Approach*, 2nd edn. McGraw-Hill Co, New York.
- Thomas, P. E. and S. Skujins. 1999. The determination of calcium and magnesium in blood serum and urine. Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia. (<http://www.varianinc.com/media/sci/apps/a-aa07.pdf>)
- Wang, J. and F. D. Provenza. 1997. Dynamics of preference by sheep offered foods varying in flavors, nutrients, and a toxin. *J. Chem. Ecol.* 23:275-288.
- Whitehead, D. C. 2000. Nutrient elements in grassland. *Soil-Plant-Animal Relationships*, pp. 148. CABI Publishing, Wallingford, UK.