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Full Length Research Paper

Iron content in forage sorghum (*Sorghum bicolor* (L.) Moench) measured on different slit widths with atomic absorption spectrometry

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Our objective was to know the right slit width for iron (Fe) concentration of forage sorghum, sorghum hybrid (*Sorghum bicolor* (L.) Moench), and also to discern which water treatment sludge (WTS) were good for ruminant's health with the feeding sorghum on the present study. The present experiment was carried out on a randomized block design with four treatments; Control, alum sludge compost, alum sludge + NPK (nitrogen, phosphorus, potassium fertilizers), alum sludge compost + NPK (nitrogen, phosphorus, potassium fertilizers). Sorghum hybrid was harvested, and iron content of it was analyzed with an atomic absorption spectrophotometer on background correction (BGC) mode. In order to analyze the iron (Fe) content of the sorghum with the spectrophotometer, three different slit widths conditions were used; 0.15, 0.20 and 0.25 nm. Absorbance and background values were obtained during the Fe analyses with the apparatus. When the background value is small, it is preferred for some trace metals' analyses. Both (AM/BS) ratio (mean of the absorbance values<AM> to the standard deviation of back ground values<BS>) and (AS<standard deviation of the absorbance values>/BS) ratio, were larger on 0.25 nm slit than those on 0.15 and 0.20 nm slit, and, from our experiment, the condition seemed better on the 0.25 nm slit for the iron analysis with the spectrophotometer. Therefore, the sorghum hybrid grown on (Alum+NPK) and on (Compost only) might be dangerous for ruminants because of their higher values than 200 mg Fe/kg DM (dry matter).

Key words: Absorbance, alum sludge, atomic absorption spectrophotometer, background, forage sorghum hybrid, iron, slit.

INTRODUCTION

In Korea, the sorghum hybrid was used as forage plant for ruminants. Fribourg (1985) described that summer annual grasses, for example sorghum, provide high quality feed in summer when well managed, but poor management results in misuse of land resources, little regrowth later in the season, and hungry livestock. All the body systems depend on minerals in order to function properly (Nelson, 1979). For ruminants, iron (Fe) was considered to be necessary more than Cu, cobalt (Co), or iodine (I) among trace elements as follows; Fe (30 to 200 mg/kg dry matter (DM)> Zn (50 mg)> Mn (40 mg)> Cu (5 to 10 mg)> I (0.12 to 0.80 mg)> Co (0.1 mg) (McDonald et al., 1985).

Until recently, (flame) atomic absorption spectroscopy (AA) was the most widely used of all atomic spectral

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methods because of its simplicity, effectiveness, and relatively low cost. The position of preeminence is now being challenged, however, by inductively coupled plasma spectroscopy (ICP), an emission method (Skoog, 1985). When a calibration curve is linear upon a range of metal's standard solutions with an atomic absorption spectrometer, they generally start the analysis of a metal. And the condition of slit width is not an important thing. While atomic absorption spectrophotometry (AA) is historically an older method than Inductively Coupled Plasma (ICP), there is some similarity between the two methods for the mineral analysis. The ratio between absorbance and background is important on ICP method, and the purpose is to get the better analysis of trace metal (Haraguchi, 1993). The slit is larger; the sensibility will be less on atomic absorption spectrophotometry. While if the slit is smaller, the signal will be insufficient for the counting or the measurement of a metal (Pinta et al., 1979).

Group VIII metals form chemical subgroups (triads) with somewhat similar behavior. Geochemical properties of the first triad, iron (Fe), cobalt (Co), and nickel (Ni), are very similar, and due to the small difference in atomic radii, they are likely to form a wide range of mixed crystals (Kabata-Pendias and Pendias, 1985). Sorghum seems particularly susceptible to Fe deficiency; thus, much of the work on cultivar differences in sorghum has been concentrated on this element (Kanwar and Youngdahl, 1985). Two previous experiments have been carried out in order to know the effects of alum sludge application (Awwarf and Kiwa, 1990) on the growth of forage sorghum (Kim et al., 1997) and the effects of alum sludge application on root growth of forage sorghum were cultivated in mountainous Kumsan district (Kim and Chang, 2000). And Cd content of some French soil on utilization of background correction (BGC) mode with an atomic absorption spectrophotometer has been analyzed (Kim et al., 2000), and analyses of trace metals of Cu, Ni, Cd and zinc (Zn) in sorghum hybrid (*Sorghum bicolor* (L.) Moench) have been also carried out (Choi et al., 2007; Kim et al., 2007; Park et al., 2009 a, b). The level of standard solution concentration was important for Cu, while the warming-up time was important for the right measuring for Ni (Park et al., 2008 a), and the burner height for Cd (Park et al., 2008 b), respectively.

When the slit is larger, the sensibility will be less on atomic absorption spectrophotometry, while if the slit is smaller the signal will be insufficient for the counting or the measurement of a metal (Pinta et al., 1979). The objective on the present study was as follows; at first, to know the right slit width with an AA spectrophotometer for Fe concentration of sorghum, and at second to discern which (sludge) treatment is better with the feeding sorghum for ruminant's health.

MATERIALS AND METHODS

The experiment was carried out in Joongbu University on from June 1993, on randomized block design with 3 replications. The treatments were Control, Alum sludge compost (Compost), Alum sludge+NPK (nitrogen, phosphorus, potassium) (Alum+NPK), Alum sludge compost+NPK (Compost+NPK). The other materials and methods for the sorghum hybrid (*S. bicolor* (L.) Moench) were already described in previous reports (Chang et al., 1993, Kim et al., 1997, Kim and Chang, 2000). The measuring method for iron (Fe) concentration of sorghum was the objective of the present experiment. The powdered plant of 0.5 g was mixed with 25 ml 1M-HCl for 18 h, and extracted through filter paper, and diluted up to 100 or 50 ml (Norin, 1979). The used atomic absorption spectrophotometer (AA-680) was 15 years old, made by Shimadzu Co., Kyoto in Japan.

The iron (Fe) content was measured on background correction (BGC) mode, after 2 to 4 h warming up as the result of Ni report (Park et al., 2008 a), burner height 6 mm, extent of Fe standard solutions ranged 0.0 to 3.0 ppm, wave length 248.3 nm, lamp currency 8 mA, acetylene flow 2.0 L/min. And the date of analysis began from 2 October, 2007 and ended 30 July, 2008. Absorbance and background values were obtained for standard solutions and for 22 samples of sorghum hybrid (*S. bicolor*). There were three slit (0.15, 0.20 and 0.25 nm) conditions.

The experimental field was situated at a mountainous site with an altitude of 260 m in Joongbu University, at *Kumsan* gun in *ChungcheongNam* do. The period of field experiment was from May to November of 1993. The field was newly established on May 26, 1993. On a ran-domized block design, there were 4 treatments with 3 replications; Control (without fertilizer or alum sludge or alum sludge compost), Alum sludge compost (Compost), Alum sludge+NPK (Alum+NPK) and Alum sludge compost+NPK (Compost+NPK) on 3 places; recently developed (higher place), medium, developed early (lower place). The alum sludge and fertilizers were applied on June 7 and June 17, 1993, respectively. The seeds of sorghum hybrid, Pioneer 931 (*S. bicolor* (L.) Moench), were sown on June 23. And the forage was harvested on November 4, 1993. The other materials and methods for the sorghum hybrid were already described in previous reports (Chang et al., 1993 b; Kim et al., 1997; Kim and Chang, 2000).

Half gram (0.5 g) of powdered plant (the powdered plant of 0.5 g) was mixed with 25 ml 1 M HCl for 18 h, and extracted through filter paper, and diluted up to 100 or 50 ml (Norin, 1979). The used atomic absorption spectrophotometer (AA 680) was 15 years old, made by Shimadzu Co., Kyoto in Japan. The Fe content was measured on BGC (background correction) mode, after 2 to 4 h warming up as the result of Ni report (Park et al., 2008 a), burner height 6 mm, extent of Fe standard solutions ranged 0.0 to 3.0 ppm (0.0, 0.3, 1.0 and 3.0 ppm), wave length 248.3 nm, lamp currency 8 mA, acetylene flow 2.0 liter/min. And the date of analysis began from 2 October, 2007 and ended 30 July, 2008. The measurement was not for emission but for absorption. Absorbance values for Fe content were obtained for standard solutions and for 22 samples of sorghum hybrid on background correction (BGC) mode.

There were three slit conditions (0.15, 0.20 and 0.25 nm). Fe content in Table 5 was calculated as follows: Fe content from the absorbance value × 200 (filled up to 100 ml), and Fe content from the absorbance value × 100 (filled up to 50 ml), respectively. The absorbance mean (AM) and background mean (BM) values of each slit treatment were shown in order to compare statistically the difference among them (Table 3), and it was done through the least significance difference (LSD) method (Snedecor and Cochran, 1980; Son and Park, 1999).

RESULTS

Calibration curves for iron (Fe) analysis on the three slit widths on background correction (BGC) mode are shown in Table 1. On the table, the mean absorbance value was 785 on (0.15 nm slit), 777.5 on (0.20 nm slit), 750 on (0.25 nm slit), respectively. The mean absorbance value decreased with the increase of slit size. And the mean background value was 12.5 on (0.15 nm slit), and it was 17.5 on (0.20 nm slit), respectively. On (0.25 nm slit) the mean value was small value of 2.5 and the background value ranged from zero to 10. Because slit might have a significant effect on linear range and slope of the calibration line would indicate the sensitivity of the Fe analysis, line equation is needed in each slit calibration results; and calibration curve of standard solutions on three slit conditions for Fe analyses in sorghum hybrid (*S. bicolor* (L.) Moench) on background correction (BGC) mode is shown in Table 2. The calibration curve for Fe standard solutions was linear on all the three slit conditions, as shown in Table 1.

Effect of slit width on background values is shown in Figure 1 during analyzing iron (Fe) content in sorghum hybrid depending upon a measuring order. In the Figure 1, the largeness of background (BG) values was different as follows; BG (0.15 nm slit) > BG (0.20 nm slit) >> BG (0.25 nm slit). This tendency of background values of samples was similar to that of absorbance values of standard solutions (Table 1). Changing patterns of absorbance of three slit treatments and the changing pattern of background were similar on 0.25 nm slit condition (Figure 2).

Table 2 shows the calibration curve on three slit conditions for iron (Fe) analyses in sorghum hybrid, Pioneer 931 (*S. bicolor* (L.) Moench). There was no significant difference among the three slit conditions. Table 3 shows the mean and standard deviation of absorbance and of background, and Table 4 shows the mean and standard deviation of absorbance and of background and the ratio among the factors. Figure 3 shows the direct Fe content from absorbance value on different slit width on measuring order (ppm). The changing pattern of Fe content was similar among the three slit conditions.

Table 5 shows the actual Fe content of sorghum hybrid (mg Fe/kg) and the correlation coefficient between the Fe values. The lower values of 85.3, 94.6, and 95.0 mg Fe were shown on 0.15 nm slit width, and the higher values of 314, 318, and 408 mg were shown on 0.25 nm slit, while the values tended to have medium- range on 0.20 nm slit. Between the three slit conditions, the Fe content had a close correlation. As it has been concluded from comparing the correlation coefficients of Cd contents among the different burner heights as follows (Park et al 2009 a); when the result of correlation coefficient of one condition were high, the condition might be best condition among the treatments. Therefore in the present study, slit 0.25 nm is the better method for Fe analysis.

Figure 4 shows the Fe content of sorghum on 0.25 nm slit on the different alum sludge application conditions. By the description of McDonald et al. (1985), Fe was considered to be necessary 30 to 200 mg/kg DM (dry matter matter) for ruminants. In the present study, the toxic level was considered above the 200 mg/kg level. Kim et al. (1997) have shown that the sorghum growth on the present study was higher on (Alum sludge compost+NPK) or (Alum sludge+NPK), while lower growth on control treatment. Therefore, the sorghum hybrid grown on (Alum sludge+NPK) at the medium place and the forage sorghum on (Alum sludge compost only) at the recently developed and at the medium places might be dangerous for ruminants with higher values than 200 mg Fe/kg DM. Also, the Fe content on (Alum sludge compost only) was higher than the content on (Alum sludge compost+NPK). As a conclusion, the (Alum sludge compost+NPK fertilizers) was the better method for ruminant's health than the (Alum sludge compost only) or the (Alum sludge+NPK fertilizers).

DISCUSSION

While simple and single step extraction procedure (using inexpensive chemicals, small sample volume, good extraction recovery, sensitivity) was not done, the results of the present study were determined with the method standard addition in order to asses the accuracy of quality control procedures (Tables 1 and 2).

In another words it can be said like this: In the traditional *Kimzang* (*Kimchi* making) in late autumn or beginning winter season in Korea, it is very important for Korean housewife to lessen water content of Chinese cabbages with salt in order to prepare the important *Kimchi* making; decrease water content to what degree? It's the quantity of salt and the time for the process. It was brine the cucumber (Perez-Diaz. in press). It is necessary to find another index. Therefore, it is considered if there is any meaningful index for the Fe analysis. In the present study it may be the decrease of background value.

Historically, atomic absorption spectrophotometry (AA) is an older method than inductively coupled plasma (ICP). The ratio between absorbance and background on AA is as important as signal/noise (S/N) ratio on ICP method. In the present study, the purpose is in order to get the better analysis of trace metal (Haraguchi, 1993). In Figure 2, absorbance and background values showed similar curves on (0.25 nm slit); that means the absorbance decreased with the advance of measuring. As shown in Figure 1, the background increased on 0.15 nm slit and (0.20 nm slit) conditions, while the absorbance had a tendency of decrease. The three (0.15, 0.20, 0.25 nm slit) conditions are on a linear form in Table 1. In this case, they would say "Now it's O.K. for the analysis!". And it was the objective of the present study; "Is the condition really appropriate for the analyst of a trace metal?".

Most monochromators are equipped with variable slits so that the effective bandwidth can be changed. The use of minimal slit widths is desirable where the resolution of narrow absorption or emission bands is needed. On the other hand, a marked decrease in the available radiant power accompanies a narrowing of slits, and accurate

Table 1. Calibration curve for iron analyses on three slit conditions on background correction (BGC) mode.

Iron (Fe) content (ppm)	Slit 0.15 (nm)		Slit 0.20 (nm)		Slit 0.25 (nm)	
	Absorbance	*Background*	Absorbance	*Background*	Absorbance	Background
0.0	10	-10	10	10	10	0
0.3	240	0	240	10	230	0
1.0	780	20	780	20	750	0
3.0	2110	40	2080	30	2010	10
Mean	785	12.5	777.5	17.5	750	2.5
Calibration curve†	Linear		Linear		Linear	

†: between absorbance value and Fe content of standard solutions.

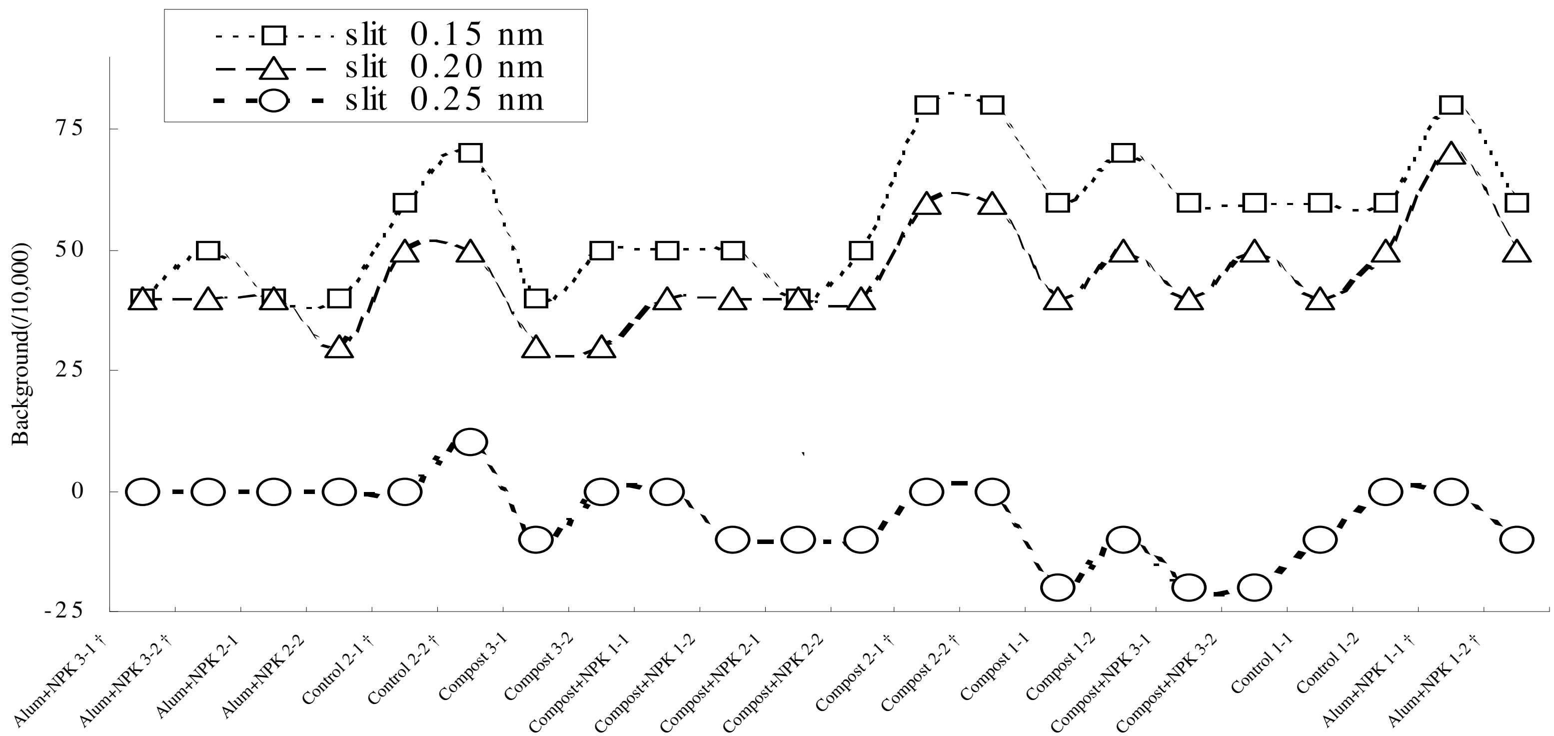

Figure 1. Effect of slit largeness on background values during analyzing iron content on background correction (BGC) mode in sorghum hybridon depending upon a measuring order from left to right. †: filled up to 50 ml, the others are up to 100 ml.

Table 2. Calibration curve of standard solutions on three slit conditions for Fe analyses in sorghum hybrid (*Sorghum bicolor* (L.) Moench) on background correction (BGC) mode.

Slit	0.15 (nm)	0.20 (nm)	0.25 (nm)
Calibration Curve &	y=14.34x - 0.05118 (r=0.9993, n= 4, p<0.01)	y=14.56x - 0.05713 (r=0.9991, n= 4, p<0.01)	Y=15.06x – 0.05486 (r=0.9992, n= 4, p<0.01)

†: the values of absorbance and Fe content are shown in Table 1; &: Calibration curve between absorbance and Fe content; here, x is absorbance(/10,000), y the Fe content (ppm) in sorghum hybrid, r regression coefficient, and n the number of the standard solutions.

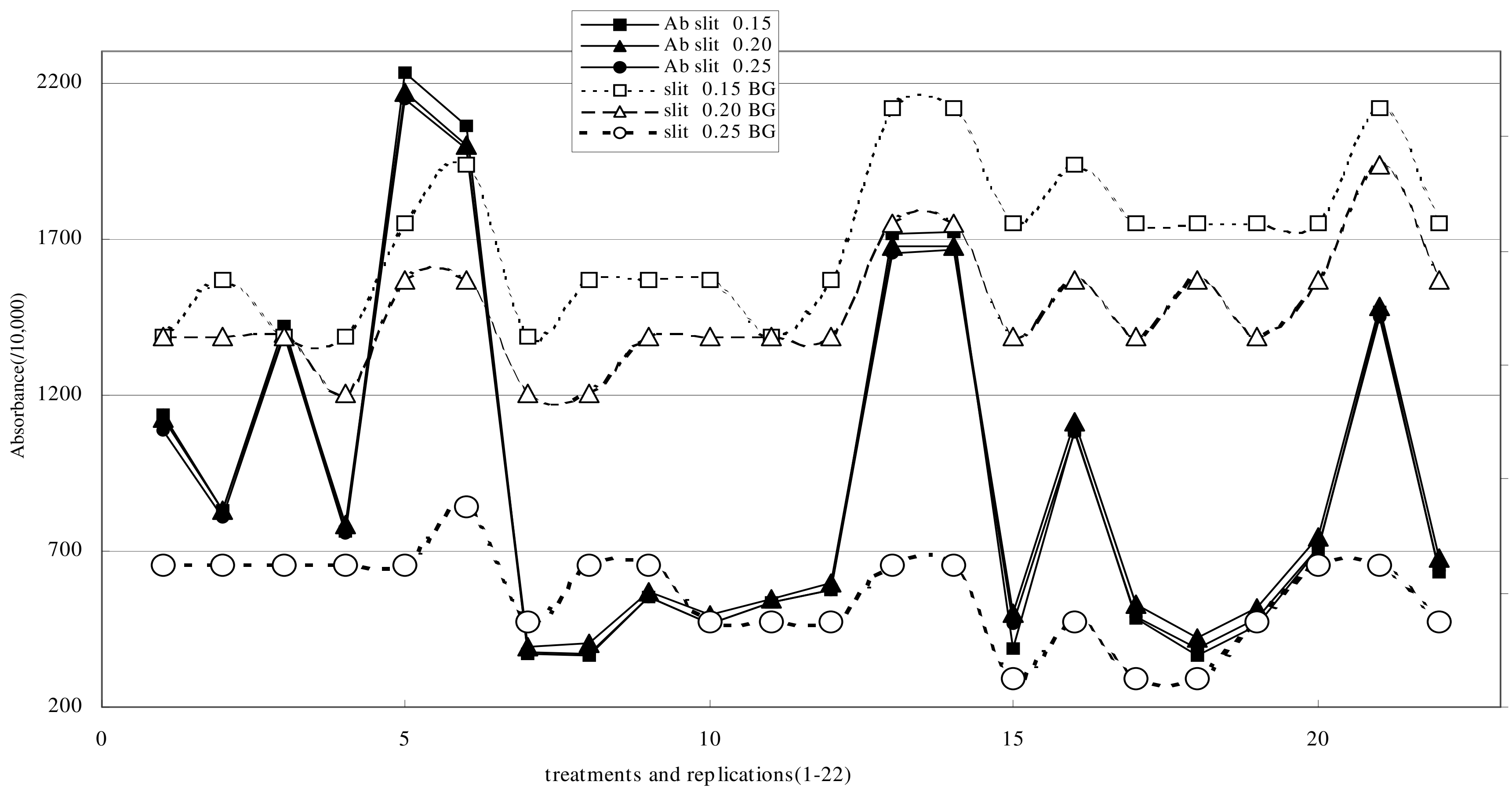

Figure 2. Effect of slit largeness on background and absorbance values during iron analyses depending upon a measuring order from left (1) to right (22) on background correction (BGC) mode.

Table 3. Mean and standard deviation of absorbance and of background on different slit widths on background correction (BGC) mode.

Parameter	Slit 0.15 (nm)	Slit 0.20 (nm)	Slit 0.25 (nm)
Absorbance mean (AM)†	924.0[ab]	938.2[a]	912.3[b]
Absorbance standard deviation (AS)	589.30	553.18	557.75
Background mean (BM)†	56.8[a]	44.5[b]	5.45[c]
Background standard deviation (BS)	13.2	10.1	8.0

†: Different characters horizontally shows the significant difference statistically ($p<0.01$), and comparing statistically the difference among them was done through the least significance difference (LSD) method.

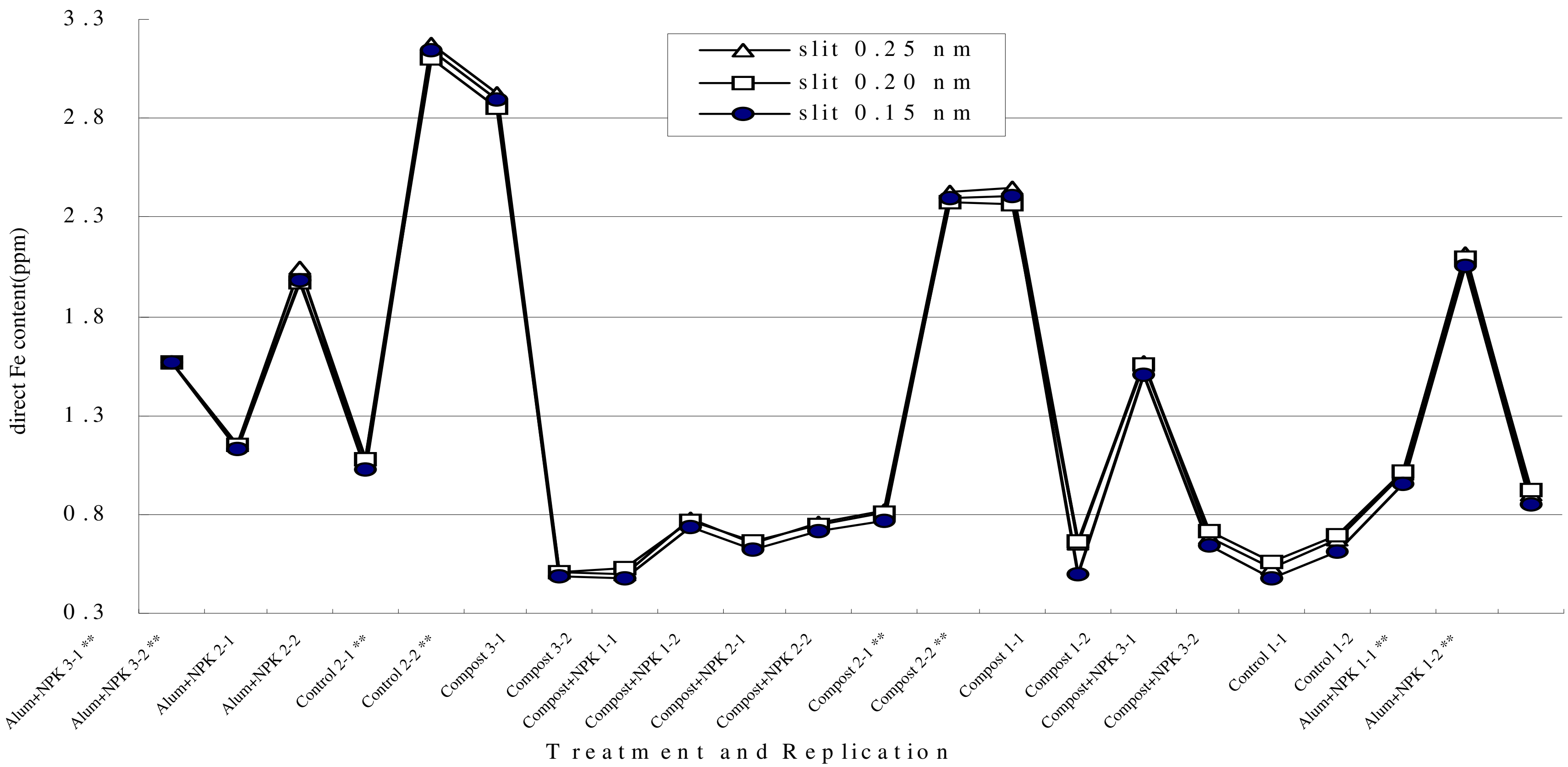

Figure 3. Direct iron (Fe) content from absorbance value on different slit width on measuring order (ppm) on background correction (BGC) mode. **: up to 50 ml, the others up to 100 ml.

Table 4. Mean and standard deviation of absorbance and of background and the ratio among the factors on different slit widths on background correction (BGC) mode.

Parameter	Slit 0.15 (nm)	Slit 0.20 (nm)	Slit 0.25 (nm)
(AM/AS)	(1.5)	(1.6)	(1.6)
(BM/BS)	(4.3)	(4.4)	(0.6)
AM/BM	16.2	21.0	167.3
(AM/BS)	(70.0)	(92.8)	(114.0)
BM/2AS (%)	4.8	4.0	0.4
(AS/BS)	(44.)	(54.)	(69.)
y=bx+a &	y 0.15= 1.1914x+ 43.11	y 0.20= 0.7905x+ 35.45	y 0.25= 0.5985x+ 1.4285
Regression coefficient(r) &	r 0.15= 0.5846 (p<0.01)	r 0.20= 0.5078 (p<0.05)	r 0.25= -0.4855 (p<0.05)

&: x is measuring order from 1(the first) to 22(the final) and y is background value, r is regression coefficient.

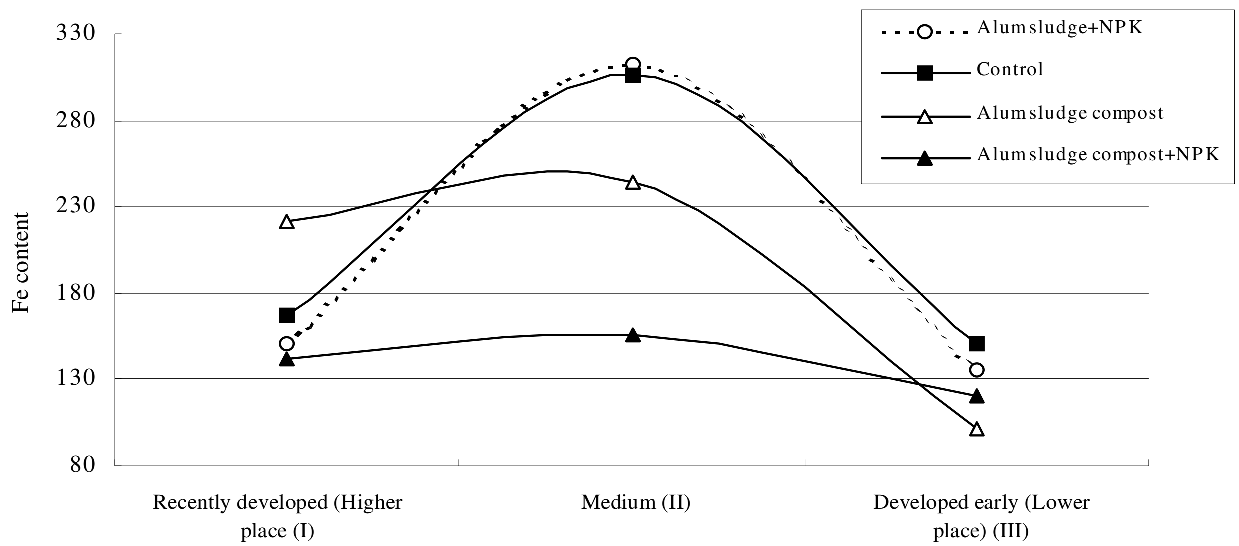

Figure 4. Iron (Fe) content of sorghum hybrid (mg/kg DW) on the condition of slit 0.25 nm on background correction (BGC) mode.

measurement of this power becomes more difficult. Thus, wider slit widths may be used for quantitative analysis than for qualitative work, where spectral detail is important (Skoog and Leary, 1992).

Therefore, from Figure 3, it was estimated favorable at0.25 nm slit width for analysis of Fe with atomic absorption spectrophotometer. The difference of content or absorbance for trace metal

Table 5. Actual iron (Fe) content of sorghum hybrid and the correlation coefficient between the Fe values on different slit widths on background correction (BGC) mode.

Actual Fe content †	Fe 1 (mg/kg)	Fe 2 (mg/kg)	Fe 3 (mg/kg)
Slit	**0.15 (nm)**	**0.20 (nm)**	**0.25 (nm)**
Control 1-1	121	138	133
Control 1-2	191	204	202
Control 2-1 &	314	310	318
Control 2-2 &	290	285	293
Alum sludge compost 1-1	100	133	130
Alum sludge compost 1-2	300	312	314
Alum sludge compost 2-1 &	240	238	243
Alum sludge compost 2-2 &	241	237	245
Alum sludge compost 3-1	96.6	102	102
Alum sludge compost 3-2	95	105	99.8
Alum sludge compost+NPK 1-1	148	154	155
Alum sludge compost+NPK 1-2	123	132	130
Alum sludge compost+NPK 2-1	142	148	150
Alum sludge compost+NPK 2-2	154	162	162
Alum sludge compost+NPK 3-1	128	142	136
Alum sludge compost+NPK 3-2	94.6	111	104
Alum sludge +NPK 1-1 &	205	210	212
Alum sludge +NPK 1-2 &	85.3	92.8	90.2
Alum sludge +NPK 2-1	396	394	408
Alum sludge +NPK 2-2	206	216	216
Alum sludge +NPK 3-1 &	157	157	157
Alum sludge +NPK 3-2 &	113	115	115
Mean	179.11	186.26	187.04
r (Fe1 : Fe2) $	0.9959		
r (Fe2 : Fe3) $	0.9994		
r (Fe1 : Fe3) $	0.9974		

†: Actual iron(Fe) content = Fe content on its absorbance value x 200 (filled up to 100 ml), and Fe content on its absorbance value x 100 (filled up to 50 ml), &: filled up to 50 ml, the others up to 100 ml, $: correlation coefficient between the Fe values on different slit widths.

was not so wide as on the different standard solutions for Cu (Choi et al., 2007), in the different warming up times for Ni (Kim et al. 2007; Park et al. 2008a), and in the burner height for Cd (Park et al., 2008b, 2009a). In another words, the difference of the Fe content among the slit treatments was small and was similar to that of Zn content among the different burner heights (Park et al., 2009b). As shown in Tables 3 and 4, the values were statistically different at 1% level. Some of the background values were under zero on (0.25 nm slit) condition. Background values on (0.15 nm slit) and on (0.20 nm slit) increased with the advance of the measuring order (Figure 1), while on (0.25 nm slit) the values decreased with a negative value of regression coefficient (Tables 3 and 4). Wider slit of 0.25 nm showed smaller absorbance in Tables 3 and 4. And these results are similar to the description of Pinta et al. (1979). The absorbance values, for the Fe analysis on (0.20 nm slit) condition, ranged higher than those on (0.25 nm slit) ($p<0.01$) (Figure 2; Tables 3, and 4). And the ratio of BM/2AS on (0.15 nm and 0.20 nm slit) conditions was greater than (0.25 nm) with the value of 4.8, 4.0 and 0.4 %, respectively. And the ratio of BS/AS was 2.2, 1.8, and 1.4 on (0.15 nm slit), on (0.20 nm slit), and on (0.25 nm slit) condition, respectively. Haraguchi (1993) wrote that the ratio of (absorbance/noise) is very important on analysis of metal with ICP method. And in the present study with the atomic absorption spectrophotometer, it was considered that the ratios of both BS/AS and BS/AS were important factors for the analysis of Fe (Tables 3 and 4). From the three conditions, therefore, the (0.25 nm slit) condition is preferable for the analysis of Fe.

Conclusion

The result of the present study showed that the Fe analysis on (0.25 nm slit) would be better than those on

(0.15 nm and 0.20 nm slit). From the three conditions therefore, the (0.25 nm slit) condition is preferable. And the (Alum sludge compost+NPK fertilizers) is the better method for ruminant's health than the (Alum sludge compost only) or the (Alum sludge+NPK fertilizers).

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