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The effect of cutting turn on the content of prussic acid and nitrate in forage sorghum

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ABSTRACT - In the sorghum plant, prussic acid and nitrate are the main anti-nutritional compounds that can harm animals if the permitted levels are exceeded. This study aimed to determine the effect of cutting sequentially on prussic acid and nitrate content in eighteen varieties of sorghum forage including four Iranian domestic and fourteen imported varieties. All varieties under the same irrigation, fertilization, light, and temperature conditions were planted. The first and second cuttings were conducted at the flowering stage, and 50 days later, respectively. According to the results, the foreign variety of FS one BMR had the highest amount of prussic acid in both cuttings but this compound level reduced from 481 ppm in the first cutting to 397 ppm in the second one. While the lowest content of prussic acid was detected in the Titan variety (163 and 37 ppm in the first and second cuttings, respectively). In the second cutting, nitrate contents were also significantly lower than those in the first one. Moreover, two varieties of Juicy sweet 2 and Juicy Sweet BMR SSH.1 recorded the maximum nitrate content (2417, and 2089 ppm, respectively) in the first cutting. By contrast, the minimum nitrate found in KFS-2 and FGCSI09 varieties by 127 and 143 ppm, respectively at the same time. Regarding the second harvesting, HFS1 and PFS-21 varieties recorded the highest nitrate content (162 and 150 ppm, respectively) whereas FGCSI12 and PHFS-27 varieties had the minimum amounts of 14 and 64 ppm, respectively. As compared with the recommended tolerable levels of prussic acid and nitrate in animal feed, the studied varieties were not toxic in the first cutting, and both compounds decreased significantly in the second cutting.

INTRODUCTION

There are major issues, such as climate change, scarcity of water sources, and drought, which limit food and feed production worldwide. More crops need to be able to survive extreme environmental conditions while producing adequate amounts of food and feed (Ogbaga et al., 2016). Sorghum [*Sorghum bicolor* (L.) Moench] is one of the appropriate crop candidates for harvesting in arid and semi-arid climates (Vinutha et al., 2017).

Sorghum ranks as the fifth most important cereal in the world after maize, rice, wheat, and barley (Getachew et al., 2016). In addition, sorghum has been suitable as a substitute for maize in both humans (Pontieri et al., 2020) and in animal nutrition (Staggenborg, 2019) due to its similar nature.

Sorghum has several advantages over other forages, such as worthy dry matter production, high productivity at second cutting, drought tolerance and better regrowth characteristics after cutting, making it an excellent summer and fall crop . However, Sorghum may have some anti-nutritional factors that limit its use as animal feed, such as prussic acid and nitrate, which are the most important compounds (Astuti et al., 2019).

In some plants, a cyanogenesis reaction occurs in which prussic acid is formed as a result of the reaction between glyco-cyanide compounds and specific enzymes. The chemical substance [(s)- parahydroxy mandelonitrile- beta-D- glocopyranozide] is a cyanogenic glucoside found in sorghum, which is synthesized from tyrosine by the action of P450s and UGT transferase enzymes (Nielsen et al., 2008). Prussic acid enters the bloodstream directly and binds with intercellular enzymes when animals are fed forages containing prussic acid. This binding prevents the transfer of oxygen into the cell and leads to death from oxygen starvation (Nielsen et al., 2008). Therefore, it is very important to monitor the concentration of prussic acid before feeding it to animals.

Prussic acid levels in plants are increased under stress conditions such as drought, frost, blight, early harvest, and excessive nitrogen fertilization (Shehab et al., 2020). The accumulation of prussic acid in the sorghum plant may limit its direct use in animal feed, as it negatively affects the health or leads to death (Ates et al., 2019; Rajasokkappan et al., 2020).

As for prussic acid's potential effects on livestock, it has been classified into three categories: 0-500 ppm as harmless (forage is generally safe and should not cause toxicity), 500-1000 ppm as hazardous (potentially toxic and forage should be fed in a limited amount) and more than 1000 ppm as toxic (very dangerous to livestock and typically causes death) (Patel et al., 2013).

Almost all forage plants contain high levels of nitrate, another anti-nutrition factor. Sorghum also has the potential to accumulate nitrate in different concentrations (Holman et al., 2019). It is worth noting that low levels of nitrate have no adverse effects on animals and even provide nutritional value by converting to nitrite and then to ammonia. In the rumen, nitrate is used to synthesize amino acids, but a diet rich in nitrates increased nitrite production. Nitrite enters the bloodstream and binds to blood hem, leading to the formation of meth-hemoglobin, which has drastic effects on animals (Sowiński & Głąb, 2018). Nitrate toxicity depends not only on the condition and age of the animals but also on the situation of the plants including growth stage, varieties, farm management, and environmental stresses such as drought, and frost (Holman et al., 2019). According to nitrate concentration, it has been classified into four groups, including less than 4400 ppm as harmless, 4400-9300 ppm safe for non-pregnant animals, 9300-15000 ppm dangerous (limit to less than 50% of dry matter ratio), and more than 15000 ppm is toxic or potentially toxic (Patel et al., 2013).

Although there have been several studies investigating prussic acid and nitrate content in the first cutting of forage sorghums, few attempts have been made to investigate the content of these compounds in the second cutting. Based on our knowledge, there is no report about the cutting effect on prussic acid and nitrate contents in a wide collection of samples. In this study, the contents of anti-nutritive factors in 18 different sorghum varieties were investigated and the influence of the cutting system on the respective prussic acid and nitrate content was evaluated.

MATERIALS AND METHODS

Sorghum varieties in this study included four domestic Iranian sorghum including Peghah, Speed-feed, Karaj Forage Sorghum 2 (KFS-2), and Karaj Forage Sorghum 18 (KFS-18). In addition, 14 imported varieties encoded CSSH.1, FGCSI09, FS one BMR, Juicy Sweet BMR SSH.1, Juicy Sweet BMR SSH.2, Titan, Silo King, PHFS-27, PFS-21, FGCSI10, Sucrose photo BMR, HFS1, FGCSI12, and juicy Sweet 2 were used. All varieties were cultivated on the research farm of the Seed and Plant Improvement Institute (SPII) in Karaj, Alborz Province, Iran.

According to the soil test, the nitrogen, phosphor, and potash requirements were determined. Fertilization with ammonium phosphate (250 kg ha^{-1}) and urea (100 kg ha^{-1}) was applied at the plowing stage when the bushes were 35-40 cm high.

The experiment was designed as a completely randomized design with three replications on the farm. There were 4 planting lines (each five m long). The distance between each line with the aside line was 60 cm. The seedlings were cultivated with 8 cm spacing on the lines. The first cut samples were made when the plants were in the flowering stage, whereas the second one was conducted after 50 days. Collected samples were transferred to the laboratory of the Animal Science Research Institute of Iran (ASRI). Then, the samples were divided into two parts, the first of which was oven-dried at 65 °C for 72 hours to calculate the dry matter (Ahn et al., 2014). To measure nitrate content, these samples were ground into powder. The samples of the other group were cut with scissors into pieces of 5 × 5 mm and used for immediate determination of prussic acid content.

Prussic acid determination

Prussic acid content was determined according to Haskins with some modifications (Haskins et al., 1984). The sample (2 g) and 100 mL of deionized water (DW) were heated in an autoclave for one hour and then filtered. Then, 10 mL of this solution was taken and extracted three times with diethyl ether. Afterward, the organic layer was decanted and heated in a steam bath at 35 °C until the ether layer almost disappeared. Subsequently, 50 mL of NaOH solution (0.1 M) was added to the extract. The UV absorbance of solutions was determined at 330 nm using a UV-spectrophotometer. The standard solution was prepared by dilution of para-hydroxyl benzaldehyde (pHB) solution in the range of 0 – 1000 ppm with 200 ppm intervals.

Nitrate determination

The nitrate content of samples was determined using Mir method (Mir, 2009). In summary, 0.5 g of powdered samples were extracted with 20 mL of water. Then, lead acetate was added to the extract to achieve a 10 % salt concentration, and the final solution was shaken using a vortex for 1 min, centrifuged at 4500 rpm (rotor 12151) for 10 min, and filtered. The obtained extract was sequentially mixed with 1 mL NaOH (5M), 1 mL magnesium chloride solution (50%), and charcoal (10-30 mg mL^{-1}). All the samples were shaken well and allowed to stand for 2-3 minutes. Then 2 mL of each extract was mixed with 2 mL distilled water and then 0.1 mL of sulfanilamide solution and 0.3 mL of HCl were added and allowed to stand for 2-3 minutes. Afterward, a 0.2 mL coupling agent was added containing citric acid (37 g), magnesium sulfate monohydrate (5 g), sulfanilamide (2 g), N-1-(naphthyl)-ethylenediamine dihydrochloride (1 g), zinc powder (1 g). After twenty min, the UV absorbance was read at 540 nm using a Spectrophotometer (BEL model UV-M51, Italy). Water was used as a blank and Potassium Nitrate (KNO_3) was used to plot as a standard curve.

Statistical analysis

Data were analyzed as the 18×2 factorial form in a completely randomized design using SAS 9.2 software (2008) via GLM procedure. The effect of the variety in each cutting was also tested using a one-way test ANOVA. The multiple comparison test was performed using the Duncan test. Differences were considered significant at the $P < 0.05$ level.

The below model was used for analyzing data:

$$Y_{ijk} = \mu + V_j + C_k + (V \times C)_{jk} + \varepsilon_{ijk},$$

where: Y_{ijk} is value in every plot, μ = population average, V_j = variety effect, C_k = cutting effect, $(V \times C)_{jk}$ = interaction effect between variety and cutting, ε_{ijk} = error effect

RESULT AND DISCUSSION

Among the studied varieties, the highest dry matter yield (20.61 ton ha^{-1}) was obtained by Siloking variety and the lowest (8.83 ton ha^{-1}) was obtained by Juicy Sweet BMR SSH.2 variety. The average of crude protein (CP) in all studied varieties was 6.357% (6.363 and 6.354 %, internal and external varieties, respectively). The mean neutral detergent fiber (NDF) in all studied varieties was 60.36% (62.06 and 59.35%, internal and external varieties, respectively). The highest amount of NDF (69.75%) was obtained in Titan variety and the lowest NDF content (54.25%) in PHFS-27 variety. The average metabolizable energy (ME) in all studied varieties was 2.41 Mcal/kg (2.33 and 2.44 Mcal/kg, dry matter (DM) basis, internal and external varieties, respectively). Juicy Sweet BMR SSH.2 and PFS-21 varieties had the highest ME levels with 2.67 and 2.64 Mcal/kg, DM basis of metabolizable energy per kilogram of dry matter, respectively, while Speed-feed and Titan varieties had the lowest ME (2.13 and 2.17 Mcal/kg, DM basis, respectively) (Gholami et al., 2022).

The analysis of the variance of prussic acid and nitrate for varieties, cuttings, and variety-cutting interaction is presented in Table 1. The effects of varieties were significant at the P < 1% level. In general, the main effects of varieties and cuttings and their interaction were significant (P <0.05). A significant interaction effect revealed that the prussic acid or nitrate content of studied varieties behaved differently in the first and second cuttings.

The mean, range, standard deviation, and coefficient variation of prussic acid and nitrate of all samples are presented in Table 2.

As for prussic acid content in the first cutting, the highest amounts were found in three varieties of FS one BMR, PHFS-27, and FGCSI10 as 481, 408, and 384 ppm, respectively. Whereas, Titan, KFS-18, and Juicy sweet 2 varieties had the lowest contents of this compound at 163, 164, and 170 ppm, respectively (Fig. 1).

Table 1. Analysis of the variance of prussic acid and nitrite (variety, cutting, and their interaction)

		HCN			Nitrate		
S.O.V	df	Mean Square	F	Sig	Mean Square	F	Sig
Variety	17	47333.013	82.821	< 0.001	838438.907	576.676	< 0.001
Cut	1	610954.898	1069.003	< 0.001	29293750.083	20148.163	< 0.001
Variety×Cut	17	4046.114	7.080	< 0.001	840254.319	577.925	< 0.001
Error	72	571.519	-	-	1453.917	-	-

S.O.V: sources of variations, df: degrees of freedom.

Table 2. The mean, range, standard deviation, and coefficient variation of prussic acid and nitrate

Variable	Mean	Range (Min-Max)	SD	CV (%)
Prussic acid	178.49	470	119.40	66.89
Nitrate	625.14	2471	735.84	117.71

S.D: Standard Deviation; CV: Coefficient of Variation

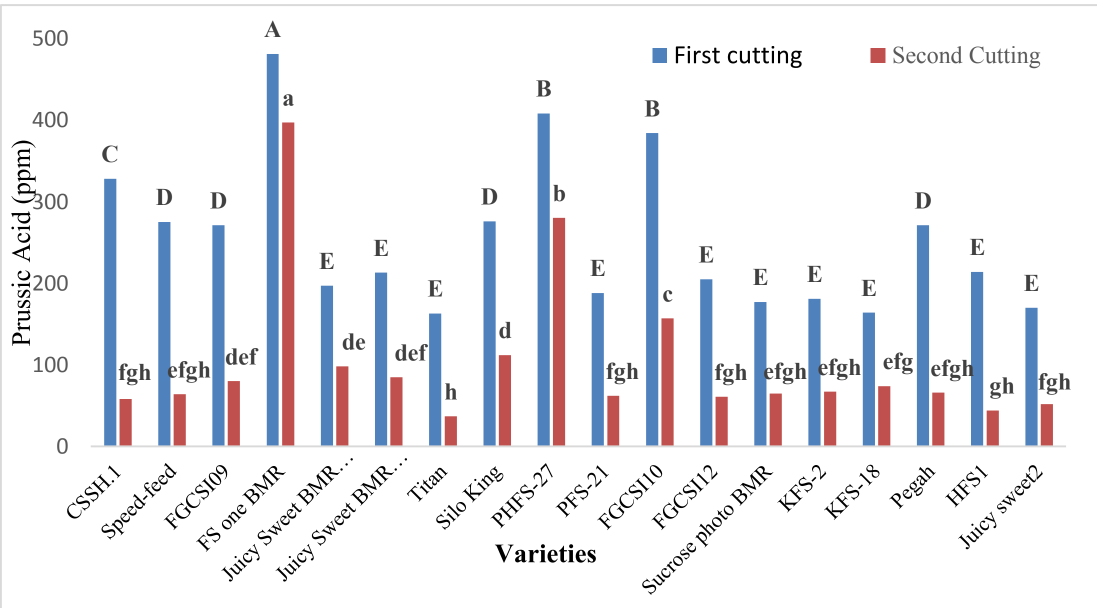

Figure 1- The effect of sorghum variety on prussic acid content in the first and second cuttings. In each series, means with different uppercase (in the first cutting) or lowercase letters (in the second cutting) are significantly different (P < 0.05)

Regarding the second cutting, the highest contents of prussic acid, like the first cutting, were found in FS one BMR, PHFS-27, and FGCSI10 varieties as 397, 280, and 157 ppm, respectively, while Titan, HFS1, and Juicy sweet 2 varieties had the lowest contents of prussic acid as 37, 44, and 52 ppm, respectively (Fig. 1).

In all varieties, prussic acid content decreased in the second cutting compared with those in the first cutting. Occasionally, this reduction was remarkable, e.g., for the CSSH.1 variety, the prussic acid content was decreased from 328 ppm in the first cutting to 58 ppm in the second cutting (more than five times lower), while for the Speed-feed variety, it was decreased from 275 ppm in the first cutting to 64 ppm in the second cutting. However, the decrease in some varieties such as FS one BMR and PHFS 27 was less extensive (from 481 to 397 ppm and from 408 to 280 ppm, in the first and second cuttings of mentioned varieties, respectively).

The main factors that influence prussic acid accumulation, as a multifactor phenomenon, reported to be plant genetic structure, plant organ (leaves have higher concentrations), fertilizer, drought, climate condition, and abiotic stresses (Ates et al., 2019; Dewi et al., 2019; Sher et al., 2014). The other factors' contributions such as plant genotype, maturation status, nutritional elements contents such as nitrogen, phosphorous, and sulfur fertilizer, temperature, day-light duration, and environmental stress also have been confirmed (Neilson et al., 2015). The data of this study suggest that the harvesting stage may be considered as an additional factor affecting prussic acid accumulation, because this factor contributed to the reduction of prussic acid levels in the second stage of cutting.

Bahrani and Deghani Ghenateghestani (2004) identified the effect of plant density and nitrogen top-dressing on yield, protein and prussic acid contents in the first and second cuttings of a sorghum forage variety named Speed-feed that had grown in two different locations of Kushkak and Zargan in Fars Province. They found that the forage prussic acid percentage was lower in the second cutting compared with that in the first cutting and the mean value of prussic acid in wet samples decreased from 150 ppm to 25 ppm in dry samples (Bahrani & Deghani Ghenateghestani, 2004). It has been suggested that the lower content of prussic acid in second cutting can be attributed to the degradation of the acid and higher metabolic activity of plant, which is due to the higher temperature during growth process.

Regarding nitrate, the results of this study showed that Juicy Sweet2, Juicy Sweet BMR SSH.1, and Sucrose photo BMR varieties had the highest levels of nitrate in the first cutting as 2417, 2089, and 2038 ppm, respectively, while the minimum contents of nitrate were found in KFS-2, FGCSI09 and Titan varieties as 127, 143 and 287 ppm, respectively (Fig. 2).

The maximum nitrate contents in the second cutting samples were found in the HFS1, PFS-21, and Juicy Sweet BMR SSH.1 varieties as 162, 150, and 146 ppm, respectively. Minimal levels were detected in FGCSI12, PHFS-27, and KFS-18 at 14, 64, and 65 ppm, respectively. In addition, there was a slight difference between the lowest and highest nitrate levels in the second cutting (14 to 162 ppm) compared to those in the first cutting (Fig. 2).

Sorghum is a forage crop that is naturally capable to accumulate nitrate, therefore, determining the nitrate content of sorghum is an important factor in order to reduce the risk of poisoning. There are a large number of published studies that focused on nutritional factors of sorghum such as DM, CP, crude fiber, NDF, and acid detergent fiber (Getachew et al., 2016; Machicek et al., 2019; Mahfouz et al., 2015). Whereas there is no attempt to study sorghum nitrate content in the second cutting. Few studies have measured the response of nitrate accumulation on cutting systems in sorghum biomass. For example, Holman et al, (2019) studied the effect of nitrogen fertilizer application rate on sorghum nitrate concentration and they found that in nitrogen fertilizer rate of 84 kg ha^{-1}, nitrate level reduced from 3181 to 78 ppm in the first to second cutting and in nitrogen fertilizer rate of 112 kg ha^{-1}, nitrate level reduced from 5484 to 143 ppm in the first to second cutting.

Similarly, it has been reported that the CP in Sudan grass forage decreased from 9.68% to 7.55% in the first to second cutting. This decrease can be explained by nitrogen availability in the first cutting due to fertilizer application, while nitrogen content decrease in the second cutting due to its mineralization (Ćupina et al., 2011).

The results of this study were consistent with other studies mentioned above. The reduced nitrate content in the second cutting was prevalent in all sorghum varieties. For the explanation of the reason for lower nitrate content in second-cutting sorghum varieties rather than the first-cutting, the Ćupina statement can be used (Ćupina et al., 2011). So it is supposed that over time, the nitrogen source became less available to sorghum, and it produced less nitrate in the second cutting because of less nitrogen absorption.

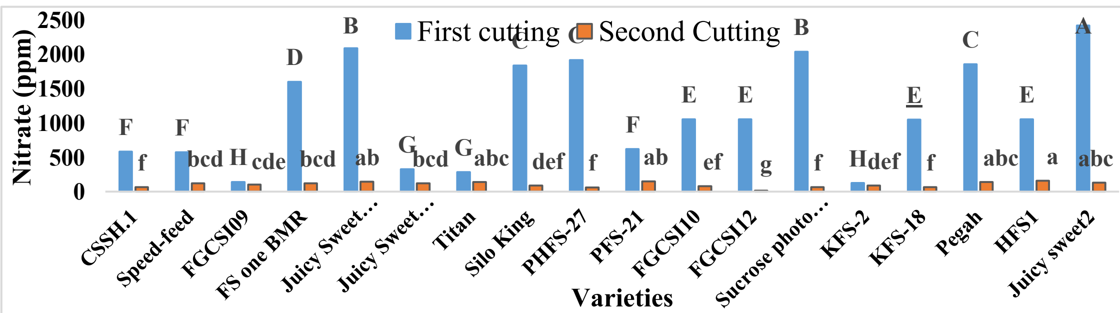

Figure 2- The effect of sorghum variety on nitrate content in the first and second cuttings. In each series, means with different uppercase (in the first cutting) or lowercase letters (in the second cutting) are significantly differ ($P < 0.05$)

CONCLUSIONS

Sorghum forages can be used as forage in semiarid climates due to their low water stress resistance. They are also used as fresh forage or silage throughout the world. When there is an adequate water source and sufficient growing season two (or even three) cutting can be harvested. Despite all the advantages of the sorghum plant, nitrates and prussic acid are considered two of the most important antinutritive components. According to the results of this study, although the prussic acid content in the first cutting was high in some varieties, it did not reach a dangerous level in any of them, while the prussic acid content in the second cutting decreased in all varieties.

Nitrate levels were also below the dangerous level in the first cutting and, interestingly, decreased more in the second cutting. The results showed that the nutritional components nitrate and prussic acid were lower in the second cutting of sorghum than in the first one. Hence, it can be concluded that the forage obtains from the second cutting of sorghum is less of a concern for livestock when it comes to using it in the diet of livestock.

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اثر نوبت چین بر غلظت اسید پروسیک و نیترات در سورگوم علوفه‌ای

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عوامل ضد تغذیه‌ای

نیترات

چکیده – اسید پروسیک و نیترات در گیاه سورگوم، مهمترین مواد ضد تغذیه‌ای هستند که می‌توانند به حیواناتی که این گیاه را بالاتر از حد مجاز مصرف می‌کنند آسیب برسانند. این مطالعه با هدف تعیین اثر نوبت چین برداشت سورگوم بر غلظت اسید پروسیک و نیترات در ۱۸ واریته این گیاه شامل ۴ واریته داخلی و ۱۴ واریته وارداتی انجام شد. همه واریته‌ها در شرایط یکسان آبیاری، کوددهی، نور و دما کشت شدند. برداشت (چین)اول در مرحله گل‌دهی و برداشت (چین)دوم ۵۰ روز بعد انجام شد. واریته خارجی FS one BMR بیشترین مقدار اسید پروسیک را در هر دو چین داشت اما مقدار آن، از ۴۸۱ پی‌پی‌ام در چین اول به ۳۹۷ پی‌پی‌ام در چین دوم، کاهش یافت. کمترین مقدار اسید پروسیک برای واریته Titan (بترتیب ۱۶۳ و ۳۷ پی‌پی‌ام در چین اول و دوم) بدست آمد. در برش دوم، محتوای نیترات نیز بطور قابل توجهی کمتر از محتوای آن در برش اول بود. در چین اول، واریته های Juicy sweet2 و Juicy sweet BMR SSH1 بیشترین مقدار نیترات (بترتیب ۲۴۱۷ و۲۰۸۹ پی‌پی‌ام) را داشتند و کمترین مقدار نیترات در چین اول در واریته های KFS-2 و FGCSI09 (بترتیب ۱۲۷ و ۱۴۳ پی‌پی‌ام) بدست آمد. اما در چین دوم واریته های HFS1 و PFS-21 بیشترین مقدار نیترات (بترتیب ۱۶۲ و ۱۵۰ پی‌پی‌ام) و واریته های FGCSI12 و PHFS کمترین مقدار نیترات (بترتیب ۱۴ و ۶۴ پی‌پی‌ام)را بخود اختصاص دادند. در مقایسه با مقادیر توصیه شده برای مصرف پروسیک اسید و نیترات در جیره دام، هیچ یک از واریته های مورد مطالعه سمّی نبودند و هر دو ترکیت در چین دوم بمقدار قابل توجهی کاهش یافتند.