

Nutrient and Poisonous Composition in the Mixed Silage of Maize and *Astragalus adsurgens* Pall. with Varied Proportions

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Abstract: Keduo 8 silage maize mixed and ensiled with *Astragalus adsurgens* Pall. at the ratio of :0, 2:1, 1:1, 1:2 and 0:1, respectively, the nutrient compositions and content of poisonous substances in all treatments determined. The result showed that with increasing proportions of *Astragalus adsurgens* Pall., the pH value and crude protein content raised while the neutral detergent fibre and acid detergent fiber had a descending trend. Mixed silage could raise the amino acid content. Aflatoxin content of treated silages were higher than those of initial herbage, respectively while the zearalenone, nitrite, nitrate, 3-Nitropropionic acid content were lower. The nutrient and amino acid content of the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. were relatively high and the contents of poisonous elements were reduced which was the ideal mixed silage treatment.

Key words: *Astragalus adsurgens* Pall., crude protein, aflatoxin, nitrite, 3-Nitropropionic acid, China

INTRODUCTION

Astragalus adsurgens Pall. is a perennial herb belonging to the genus of *Astragalus* in leguminosae. It is an important forage legume distributed throughout China and Mongolia as well as in some areas of Korea, Russia and Japan.

Astragalus adsurgens Pall. has a strongly developed root system with luxuriant foliage and spreading branches and is an ideal water and soil conservation plant. Its stems and leaves are the green fodder for cattle and other farm animals (Zhang *et al.*, 2009). As found in other legumes, however, it is nutritionally deficient in methionine that is an essential amino acid for animal nutrition and must be acquired from the food supply (Bai *et al.*, 2009). In addition, being a forage grass, it smells and tastes awful due to the nitro compound content. During haymaking in the Autumn because of its large defoliation, thick and stiff stem and inferior quality, the intake rate of livestock is only 60%. Therefore, not only is the forage grass resource wasted but also the economic value of *Astragalus*

adsurgens Pall. is decreased. Studies have reported that not all *Astragalus* plants in leguminosae can be used as forage because some of them contain toxins would cause acute or chronic poisoning for livestock. *Astragalus* and *Oxytropis* species have the highest potential to cause serious harm for the livestock among all the toxic plants in North America. Stermitz (1969, 1972) isolated and identified miserotoxin, namely 3-Nitro-1-propyl- β -D-glucoside from *A. misen* Var. *oblongifolius* and it could be decomposed into virulent 3-Nitro-1-propanol.

Stermitz and Harlow (1975) isolated and identified Hiptagin (1, 2, 3, 4-tetra-3-nitro-propanoyl- β -D-glucopyranoside) and Cibarian (1.6-di-(3-nitropropanoyl)-1- β -D-glucopyranoside). These nitro compounds could be metabolised into 3-Nitropro-Piomie Acid (3-NPA) while their toxicity per gram of plant tissue are approximately equal, causing acute or chronic poisoning of livestock. Chronic poisoning would bring about flaccid hindquarter with the following symptom complex: asthenia universalis, hindquarter disequilibrium, tremors, diarrhea, emaciation, dyspnoea, prostration and at last heart and lung failure

and death. Acute poisoning would cause the animal to die within 2-24 h after ingestion. The poisoning happens mainly in ruminant animals, rarely in equids (Stermitz and Harlow, 1975).

By the end of 2006, 27 genera and 37 species of fungus had been found in *Astragalus adsurgens* Pall. all over the world. They caused 22 kinds of fungal diseases in which 25 genera and 34 species of fungus in the stem and leaf caused 20 kinds of diseases and 3 genera and 7 species of fungus in the root caused 3 kinds of diseases (Reddy *et al.*, 2009). Most of the fungi of *Astragalus adsurgens* Pall. are probably metatrophic bacteria such as *Stysanus*, *Stachybotrys*, *Aspergillus*, *Rhizopus* and *Penicillium*. Aflatoxin is the metabolite of *Aspergillus flavus* and *Aspergillus parasiticus* which is the strongest chemical carcinogen found so far and has intense teratogenic and mutagenic action (Mussaddeq *et al.*, 2000; Shahidi Bonjar, 2004; Aryantha and Lunggani, 2007; Sawale and Gosh, 2008; Youssef *et al.*, 2008; Gachomo and Kotchoni, 2008). Because of the toxic compounds in *Astragalus adsurgens* Pall and the difficulty in making high quality silage, *Astragalus adsurgens* Pall. and Keduo 8 *Zea mays* were mixed and ensiled to study the method for reducing the content of poisonous substances and explore the optimal ratio of mixed silage and thus provide scientific evidence for using *Astragalus adsurgens* Pall. in ruminant fodder.

MATERIALS AND METHODS

Site and soil details: The research was performed at Linxi (118.02°N, 43.62°E, 900 m altitude) located in Inner Mongolia of China. The vegetation season was from May to October. Total precipitation was 365 mm. Average temperature was 4.3°C. The major soil characteristics based on the method described by Rowell (1996) were as follows: the soil type was chestnut soil, organic matter was 10.20 mg kg⁻¹ by the Walkley-Black Method; total salt was 3.20 mg kg⁻¹ rapid available phosphorus was 50.07 mg kg⁻¹, rapid available potassium was 160.73 mg kg⁻¹, pH was 7.20 in soil saturation extract.

Experimental design: Keduo 8 was a silage maize which planted a large number of area in China. There were 5 treatments: single maize silage, 2/3 maize and 1/3 *Astragalus adsurgens* Pall. mixed silage, 1/2 maize and 1/2 *Astragalus adsurgens* Pall. mixed silage, 1/3 maize and 2/3 *Astragalus adsurgens* Pall. mixed silage and single *Astragalus adsurgens* Pall. silage.

Ensiling: Maize sowed in May 2008 was harvested at kernel milky maturity stage. *Astragalus adsurgens* Pall. was harvested after three growing years at the bud stage.

Then, they were chopped using a hay cutter to about 2 cm. The chopped forage was mixed and divided into equal portions (600 g portion⁻¹) for application of 5 treatments. Forage from each treatment was packed into 5 polyethylene bags and sealed. At the end of the 75 days ensiling period the silages were sampled for the analysis of nutritive value and poisonous compounds of maize and *Astragalus adsurgens* Pall. mixed silage.

Analyses: Silage samples were analysed using standard procedures of AOAC (2000): DM (920.36) after drying at 105°C for 24 h; ash (923.03) by igniting at 550°C for 3 h in a muffle furnace. Neutral Detergent Fibre (NDF) was analysed by a method of Van Soest *et al.* (1991). Acid Detergent Fibre (ADF) was analysed sequentially on the same sample by a method of AOAC (2000; 973.18). Crude Protein (CP) were determined by a Rapid N cube Analyzer (Germany, Eementar company). Water Soluble Carbohydrates (WSC) were determined using the Anthrone Method (Owens *et al.*, 1999). Amino acid were determined by a High Speed Amino Acid Analyzer (Hitachi 835-50). Aflatoxin and zearalenone were determined by Vicam Mycotoxin Test System (AOAC, 2000; 993.31). Nitrite and nitrate were determined by Ion-exchange chromatography (EN 12014-4-2005). 3-NPA was determined by a Flame Ionization Detector (FID), Boron trifluoride-methanol derivatization and 50-120°C programmed temperature rising method (gas chromatograph GC2010 from Shimadzu Corporation, Japan).

Statistical analysis: The statistical analysis was conducted with the aid of the Statistics Software SAS8.1. This consisted of an ANOVA (proc GLM) and a subsequent comparison of the mean values. Means were separated by LSD at the 5% level of significance.

RESULTS

Nutrient composition: The ash content of *Astragalus adsurgens* Pall. single silage was the highest which was significantly different from other treatments ($p < 0.05$). Mixed silage with increasing proportions of *Astragalus adsurgens* Pall. showed an increasing trend for crude protein and those of the maize single silage and 2/3 maize and 1/3 *Astragalus adsurgens* Pall. mixed silage treatment were significantly lower than others ($p < 0.05$) with a crude protein content of *Astragalus adsurgens* Pall. up to 15.03 in the DM. With increasing proportions of *Astragalus adsurgens* Pall., NDF showed a decreasing trend which suggested that the mixed silage could decrease the fiber content of feeds and increase digestibility (Table 1).

Table 1: pH and nutrient composition of maize and *Astragalus adsurgens* Pall. mixed silage

Treatments	pH	DM (%)	DM (%)				
			Ash	CP	WSC	ADF	NDF
Maize	3.50 ^a	25.07 ^a	7.16 ^a	7.28 ^a	3.82 ^c	30.26 ^a	54.23 ^c
2/3 Maize +1/3 <i>Astragalus adsurgens</i> Pall.	4.16 ^b	26.08 ^a	6.12 ^a	10.72 ^b	2.32 ^b	28.67 ^a	50.33 ^c
1/2 Maize +1/2 <i>Astragalus adsurgens</i> Pall.	4.55 ^b	27.37 ^{ab}	7.05 ^a	14.69 ^c	2.31 ^b	25.93 ^a	41.92 ^{bc}
1/3 Maize +2/3 <i>Astragalus adsurgens</i> Pall.	4.68 ^c	27.65 ^b	7.64 ^a	14.86 ^c	2.28 ^b	25.88 ^a	41.93 ^{bc}
<i>Astragalus adsurgens</i> Pall.	4.99 ^c	30.48 ^c	8.10 ^b	15.03 ^c	1.87 ^a	23.98 ^a	32.51 ^a

pH = pH-value; DM = Dry Matter; CP = Crude Protein; WSC = Water-Soluble Carbohydrates; ADF = Acid Detergent Fiber; NDF = Neutral Detergent Fiber; ^{a-c}Means in the same column with different superscript letters differ significantly (p<0.05)

Table 2: Amino acid content of maize and *Astragalus adsurgens* Pall. mixed silage

Treatments	DM (%)							
	LYS	TRY	PHE	MET	THR	ILE	LEU	VAL
Maize	0.0887 ^a	0.0233 ^a	0.0898 ^a	0.0443 ^b	0.0739 ^a	0.0976 ^a	0.1498 ^a	0.1351 ^a
2/3 Maize +1/3 <i>Astragalus adsurgens</i> Pall.	0.0827 ^a	0.0199 ^b	0.0866 ^b	0.0294 ^b	0.0536 ^b	0.0865 ^b	0.1280 ^a	0.0997 ^b
1/2 Maize +1/2 <i>Astragalus adsurgens</i> Pall.	0.1395 ^b	0.0695 ^c	0.1863 ^c	0.0351 ^b	0.1471 ^c	0.1888 ^{cd}	0.2977 ^a	0.2193 ^c
1/3 Maize +2/3 <i>Astragalus adsurgens</i> Pall.	0.1287 ^c	0.0619 ^c	0.1517 ^c	0.0254 ^b	0.1182 ^c	0.1470 ^c	0.1300 ^a	0.1791 ^c
<i>Astragalus adsurgens</i> Pall.	0.1753 ^d	0.0810 ^d	0.1648 ^c	0.0197 ^a	0.1345 ^d	0.1619 ^d	0.2536 ^c	0.1928 ^c

LYS = Lysine; TRY = Tryptophan; PHE = Phenylalanine; MET = Methionine; THR = Threonine; ILE = Isoleucine; LEU = Leucine; VAL = Valine; ^{a-c}Means in the same column with different superscript letters differ significantly (p<0.05)

Amino acids: With increasing proportions of *Astragalus adsurgens* Pall., all analyzed amino acids showed an increasing trend. Except for leucine, the amino acid content of maize silage and mixed silage of 2/3 maize and 1/3 *Astragalus adsurgens* Pall. was significantly lower than others (p<0.05), *Astragalus adsurgens* Pall. silage was the highest which indicated that the mixed silage would increase the amino acid content of maize silage. The 8 kinds of amino acid content and the total amino acid content of the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. were higher than other two mixed silage treatments. The methionine content of single maize silage was up to 0.0443% (Table 2), higher than other treatments while that of single *Astragalus adsurgens* Pall. silage was 0.0197% and significantly different from other treatments (p<0.05), suggesting that methionine in *Astragalus adsurgens* Pall. was scarce, mixed silage with maize could increase its methionine content.

Aflatoxin and zearalenone: The aflatoxin content of each treatment was higher than its herbage and the difference between the mixed silage of 1/3 maize and 2/3 *Astragalus adsurgens* Pall. and single *Astragalus adsurgens* Pall. silage was significant (p<0.05). This suggested that ensiling increased the aflatoxin content of the herbage. The aflatoxin content of *Astragalus adsurgens* Pall. silage was the highest, up to 8.212 µg kg⁻¹ (Table 3). The zearalenone content of the silage was significantly lower than that of herbage. The difference between the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. and the mixed silage of 1/3maize and 2/3 *Astragalus adsurgens* Pall. was significant (p<0.05) which suggested that silage

Table 3: Aflatoxin and zearalenone content of maize and *Astragalus adsurgens* Pall. mixed silage

Treatments	Aflatoxin (µg kg ⁻¹)		Zearalenone (µg kg ⁻¹)	
	Herbage	Silage	Herbage	Silage
Maize	1.24 ^a	2.84 ^a	1.32 ^a	0.92 ^b
2/3 Maize +1/3 <i>Astragalus adsurgens</i> Pall.	0.14 ^b	2.20 ^a	0.61 ^a	0.54 ^b
1/2 Maize +1/2 <i>Astragalus adsurgens</i> Pall.	0.23 ^b	5.40 ^a	1.70 ^a	0.96 ^{bc}
1/3 Maize +2/3 <i>Astragalus adsurgens</i> Pall.	0.68 ^b	5.80 ^{ab*}	0.88 ^a	0.34 ^{ab*}
<i>Astragalus adsurgens</i> Pall.	2.48 ^c	8.21 ^{b*}	0.70 ^a	0.42 ^a

^{a-c}Means in the same column with different superscript letters differ significantly (p<0.05). *Denotes significant differences between herbage and silage (p<0.05)

Table 4: Nitrite and nitrate content of maize and *Astragalus adsurgens* Pall. mixed silage

Treatments	Nitrite (mg kg ⁻¹)		Nitrate (mg kg ⁻¹)	
	Herbage	Silage	Herbage	Silage
Maize	0.174 ^b	0.057 ^a	456.32 ^a	343.25 ^a
2/3 Maize +1/3 <i>Astragalus adsurgens</i> Pall.	0.172 ^b	0.066 ^{ab*}	504.11 ^b	389.30 ^a
1/2 Maize +1/2 <i>Astragalus adsurgens</i> Pall.	0.169 ^b	0.067 ^{ab*}	473.24 ^a	369.27 ^{ab*}
1/3 Maize +2/3 <i>Astragalus adsurgens</i> Pall.	0.198 ^b	0.078 ^b	486.35 ^a	382.74 ^a
<i>Astragalus adsurgens</i> Pall.	0.186 ^b	0.117 ^c	529.29 ^a	353.12 ^{ab*}

^{a-c}Means in the same column with different superscript letters differ significantly (p<0.05). *Denotes significant differences between herbage and silage (p<0.05)

could reduce the zearalenone content. The zearalenone content of the mixed silage of 1/3 maize and 2/3 *Astragalus adsurgens* Pall. and *Astragalus adsurgens* Pall. silage were significantly lower than other three treatments (Table 4).

Nitrite and nitrate: The silage reduced the nitrite content of each treatment, the mixed silage of 1/2 maize and 1/2

Table 5: The 3-Nitropropionic acid content of maize and *Astragalus adsurgens* Pall. mixed silage

Treatments	3-Nitropropionic acid (mg kg ⁻¹)	
	Herbage	Silage
Maize	0.091 ^a	0.054 ^a
2/3 Maize +1/3 <i>Astragalus adsurgens</i> Pall.	3.654 ^b	1.375 ^{ab*}
1/2 Maize +1/2 <i>Astragalus adsurgens</i> Pall.	3.932 ^b	1.283 ^{b*}
1/3 Maize +2/3 <i>Astragalus adsurgens</i> Pall.	4.871 ^c	1.879 ^{b*}
<i>Astragalus adsurgens</i> Pall.	7.270 ^c	2.604 ^{c*}

^{a-c}Means in the same column with different superscript letters differ significantly (p<0.05); *Denotes significant differences between herbage and silage (p<0.05)

Astragalus adsurgens Pall. was significant (p<0.05). The nitrite content of *Astragalus adsurgens* Pall. silage and the herbage were all higher than that of the other treatments (Table 5). The silage reduced the nitrite content of each treatment. Nitrite content of the herbage and the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. were significantly different (p<0.05) while that of single *Astragalus adsurgens* Pall. silage was significantly higher than other treatments suggested that *Astragalus adsurgens* Pall. was sensitive to nitrate and nitrite.

3-Nitropropionic acid: The 3-NPA of the maize silage treatment not significantly different from its herbage (p>0.05) while that of other treatments were significantly lower than their herbage (Table 5). This suggested that silage reduced the 3-NPA content. With increasing proportions of *Astragalus adsurgens* Pall., both herbage and silage of 3-NPA content increased while the 3-NPA content of the herbage and silage from the mixed silage of 2/3 maize and 1/3 *Astragalus adsurgens* Pall. and the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. were not significantly different.

DISCUSSION

Astragalus adsurgens Pall. like other perennial legumes was a difficult material for ensiling due to its high buffering capacity (474 ME kg⁻¹ DM) and low water soluble carbohydrate content (23.4 g kg⁻¹ DM). The water soluble carbohydrate content was one of the most important factors and the content of water soluble carbohydrate with reach 80-100 g kg⁻¹ DM for proper ensiling (Yu *et al.*, 2008). While the sugar content of maize was relatively high and the sugar content of maize and *Astragalus adsurgens* Pall. mixed silage could meet the requirement of pasture silage. Leguminous pasture silage has a strict requirement for moisture content (not exceeding 60-70%). It was measured that the moisture content of *Astragalus adsurgens* Pall. fresh grass was 66.52% and that of maize was around 75% therefore, the mixture could meet the requirement of ensiling for moisture.

The pH value of *Astragalus adsurgens* Pall. and maize mixed silage treatments was significantly lower than that of single *Astragalus adsurgens* Pall. silage (p<0.05) which indicated that *Astragalus adsurgens* Pall. was not suitable for single silage. Moreover, the crude protein content of maize was low and the mixed silage with *Astragalus adsurgens* Pall. could significantly increase the crude protein content of the silage. During the process of the mixed silage of whole-plant maize and *Astragalus adsurgens* Pall., the merit of high protein content in *Astragalus adsurgens* Pall. was brought into full play, the difficulty of single leguminous pasture silage and great loss of nutrition could be resolved, the best complementation of energy and protein was realized and the utility value of pasture was also improved (Gao, 2003). Zhang (2003) reported that the crude protein and ash content of maize stover dry matter after ensiling were increased by 25.51 and 9.04% and the NDF and ADF content dropped 16.37 and 23.66%, meanwhile the effective degradability *in vivo* of DM and NDF were raised by 20.72 and 10.43% which suggested that ensiling could increase the digestibility of maize straw in the rumen. In this experiment with increasing proportions of *Astragalus adsurgens* Pall., crude protein and ash content showed an increasing trend while NDF and ADF content were decreasing which suggested that the mixed ensiling would help to increase the digestibility of silage and was consistent with Zhang's experiment result.

Astragalus adsurgens Pall. has high protein content and is rich in essential amino acids while maize has low protein content, especially the lysine content thus protein fodder is needed for feeding livestock. In this experiment, with the proportion of mixed *Astragalus adsurgens* Pall. increasing, the lysine content of each mixed silage treatments was 4.5, 57.2 and 45.0% higher than that of single maize silage, respectively. Meanwhile, the content of other amino acids and the total value in the mixed silage treatments of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. as well as 1/3 maize and 2/3 *Astragalus adsurgens* Pall. were higher than those of single maize silage which fully demonstrated that the nutrient value of mixed silage was more comprehensive and richer than that of single maize silage. However, the content of each kind of amino acid in the mixed silage treatment of 2/3 maize and 1/3 *Astragalus adsurgens* Pall. was lower than that of the single maize silage which might result from the great protein loss with this ratio causing amino acid degradation and this warrants further investigation.

Ao and Chen (2008) evaluated the detection level and distribution characteristics of 6 main mycotoxins (aflatoxin, ochratoxin, fumitremorgin, zearalenone, vomitoxin, T-2toxin) of the feedstuffs in China in 2006 and the first half of 2007 and found that in the silage, except the detection level of fumitremorgin was 88.2%, others

were all 100%. Zearalenone could cause hyperestrinism syndrome of human and animal (Songsermsakul *et al.*, 2006; Binder *et al.*, 2007). China formulated the limit at 60 $\mu\text{g kg}^{-1}$ for human wheat and maize consumption, 500 $\mu\text{g kg}^{-1}$ for the feeds (Collins *et al.*, 2006). Studies have shown that adding 30-40% of methionine over the NRC standard and raising the content of Vitamin A, D, K and comprehensive nutrients will reduce the toxic effects of zearalenone (Creppy, 2002). In this experiment, the zearalenone content of *Astragalus adsurgens* Pall. silage was 0.0197 $\mu\text{g kg}^{-1}$ and the methionine content of three mixed silage treatments of maize and *Astragalus adsurgens* Pall. was 0.0294, 0.0351 and 0.0254 $\mu\text{g kg}^{-1}$, respectively which were all higher than that of the single *Astragalus adsurgens* Pall. silage therefore, this is effective for reducing the zearalenone content and its toxicity.

The nitrate itself in the feeds has no toxicity for the animal and it becomes toxic only after being converted into nitrite (Sar *et al.*, 2005; Menneer *et al.*, 2008). After the feeds with high content of nitrite was eaten and absorbed by the livestock, the oxygen cycle in the blood of them could be affected, causing disease to the livestock and even death in the severe case. It was reported that the lethal dosage of nitrite nitrogen was 6-34 mg kg^{-1} weight for livestock like cow, sheep, pig, rabbit and dog, etc. (Yang *et al.*, 2002). Zhang *et al.* (2008) proved that the *Astragalus adsurgens* Pall. in China was a low-toxic plant of *Astragalus* genus and feeding it to the ruminants would neither cause anatomic disease or tissue lesion and nor affect the growth, development, reproduction and meat quality. In this experiment, mixed silage samples with different ratio were tested and it was found that the nitrite content of 1/3 maize and 2/3 *Astragalus adsurgens* Pall. mixed silage was 0.198 mg kg^{-1} , the highest before ensiling that of the single *Astragalus adsurgens* Pall. silage was 0.117 mg kg^{-1} , the highest after ensiling.

Li (2007) adopted the same method as that mentioned in this study to determine the 3-NPA content of five samples including Wutai wild *Astragalus adsurgens* Pall., Henan *Astragalus adsurgens* Pall., Shanxi *Astragalus adsurgens* Pall., Pengyang premature *Astragalus adsurgens* Pall. and Zhongsha 1 hybrid *Astragalus adsurgens* Pall.

CONCLUSION

It was concluded that the 3-NPA content ranged from 1.21-11.30 mg kg^{-1} . In this study, the 3-NPA content of all treatments before ensiling was in this range and 3-NPA was determined at a low level in the maize before and after

ensiling. Except for the single maize silage, the 3-NPA content of treatments after ensiling decreased significantly which suggested that ensiling was effective in reducing the 3-NPA content in the plant of *Astragalus adsurgens* Pall. and that of the mixed silage of 1/2 maize and 1/2 *Astragalus adsurgens* Pall. as well as the single *Astragalus adsurgens* Pall. silage decreased the most, 67.37 and 64.18%, respectively.

ACKNOWLEDGEMENTS

The researchers acknowledge the financial supported by the earmarked fund for Modern Agro-industry Technology Research System. We also thank the staves of Station of Grassland Work Station of Linxi who helped to plant the field experiment. Thanks to Grant Kaye for his valuable and insightful comments which greatly improved the manuscript.

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