



## Reducing the alkaloids content improves the edibility of mulberry as animal feed

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### ABSTRACT

The mulberry latex has high concentration of sugar-mimic alkaloids. This study indicated that the alkaloids caused animals diarrhea and weight loss. Washing away the latex and then a mild fermentation with *Lactobacillus acidophilus* improved the edibility of mulberry as animal feed.

**Key words:** Mulberry (*Morus alba* L.), alkaloids, latex, fermentation, feed.

**Abbreviations:** 1-DNJ, 1-deoxynojirimycin.

### INTRODUCTION

Mulberry (*Morus alba* L.) is one of the most highly leaf-yielding trees. As newly developed forage crop, mulberry has high content of protein as well as, bioactive components which improve animals immunity (Islam et al., 2008) and reduce the use of antibiotics in aquaculture, but natural mulberry is usually poor with palatability and cause weight loss of animals (Al-kirshi et al., 2010; Li et al., 2012).

Mulberry trees are characterized by latex secretion. The latex contains protein and three kinds of alkaloid: 1, 4-dideoxy-1, 4-imino-d-arabinitol, 1-deoxynojirimycin (DNJ) and 1, 4-dideoxy-1, 4-imino-D-ribitol (Konno et al., 2006). These alkaloids are  $\alpha$ -glucosidase inhibitors (Asano et al., 2001).  $\alpha$ -glucosidase catalyze the hydrolysis of glycosides or oligosaccharides into glucose and  $\alpha$ -glucosidase inhibitors inhibit the process of hydrolysis. These alkaloids are soluble in water and account for about 2.46% of wet weight of the latex, which maybe the highest in plant kingdom (Konno et al., 2006).

This study indicated that the mulberry latex alkaloids cause diarrhea in animals. After washing away the latex with water and a mild fermentation with *Lactobacillus acidophilus*, the alkaloids content decreased and mulberry palatability was improved while animals showed fewer diarrheas with this mulberry feed.

### MATERIALS AND METHODS

Mulberry cultivar Husang 32 was planted in Institute of

Economic Crops, Hubei Academy of Agricultural Sciences, China. Chickens from Hongshanji and piglets Dabai were reared in the Institute of Animal Husbandry and Veterinary Sciences, Hubei Academy of agricultural sciences, China. *Lactobacillus acidophilus* was obtained from China center for culture collection.

#### Collecting of latex alkaloids and protein

Mulberry latex were collected as described as Konno et al. (2006), and then removed of protein by ethanol precipitation (70% ethanol) to obtain alkaloids. The precipitated protein was collected and stored at 4°C.

#### Washing away of mulberry late

Fresh leaves were cut into strips (3 to 6 mm in width), and soaked in tap water for 30 min and thereafter removed from the water.

#### Chickens feeding with mulberry alkaloids meal

3-month-old and weighing about 500 g chickens were divided into two groups (each group having 5 chickens). The chickens were fed with 10 g/day normal feed per chicken for 5 days and one group fed with alkaloids meal

**Table 1:** Effect of mulberry latex ingredients on chicken defecation.

Normal meal		Mulberry latex protein meal		Normal meal		Mulberry latex alkaloids meal	
1 <sup>th</sup> day	33.7	6 <sup>th</sup> day	5.3	1 <sup>th</sup> day	5.2	6 <sup>th</sup> day	17.5
2 <sup>th</sup> day	55.6	7 <sup>th</sup> day	4.1	2 <sup>th</sup> day	4.7	7 <sup>th</sup> day	19.4
3 <sup>th</sup> day	33.9	8 <sup>th</sup> day	4.6	3 <sup>th</sup> day	3.5	8 <sup>th</sup> day	17.8
4 <sup>th</sup> day	44.2	9 <sup>th</sup> day	5.5	4 <sup>th</sup> day	4.6	9 <sup>th</sup> day	18.5
5 <sup>th</sup> day	33.5	10 <sup>th</sup> day	4.0	5 <sup>th</sup> day	5.3	10 <sup>th</sup> day	17.3
Average	44.2	Average	4.7	Average	4.6	Average	18.1***

Chickens watery unformed feces rate (%). \*\*\*P < 0.001.

(0.1 g mulberry latex removed from protein was mixed into 10 g normal feed) for 5 days. The other group was fed with protein meal (protein collected from 0.1 g mulberry latex mixed into 10 g normal feed) for 5 days. The defecation of chickens was observed and the significantly increased watery unformed feces rate taken as the symptom of diarrhea.

### Mulberry inhibition on starch hydrolysis

200 mg of mulberry leaves (dry) were ground and mixed with 1 ml of 0.02 mol /L HCl at 40°C for 60 min and centrifuged at 10000 r/min for 10 min. The extract was obtained by collecting the supernatant and stored at 60°C for 30 min to exclude HCl. The enzyme activity of amylase was determined according to the instructions of amylase assay kit (Wuhan Life Origin Biotech Co., Ltd.): 100 U of enzyme was added into 250 µl reaction system with 30 µl of mulberry extract; 30 µl of ddH<sub>2</sub>O served as a control (Original enzyme activity). The formula used for calculating the inhibition rate is given as:

$$\text{Inhibition rate} = \frac{\text{Original enzyme activity} - \text{Enzyme activity in samples}}{\text{Original enzyme activity}} \times 100\%$$

### DNJ (alkaloid) and protein content

Mulberry DNJ content assay was performed according to the method as described by Shi et al. (2006). The assay of protein content was in line with Chinese national standards determination 5009.10.

### Fermentation

The mulberry leaves were sprayed with *L. acidophilus* in a container, fermented for 48 h at 30°C. 1-month-old piglets weighing about 25 kg were fed with mulberry meal and piglets divided into four groups (each group having 5 piglets), and fed with normal feed for 7 days and thereafter, separately fed with different meals for 12 days. Group A: mulberry meal (normal feed mixed with 10% dry mulberry

powder); Group B: mulberry meal (10% mulberry removed latex); Group C: mulberry meal (10% mulberry removed latex and then fermented); Group D: normal feed. The piglets eating habit and defecation were observed and the weight gain recorded.

## RESULTS

### Chickens showed diarrhea after fed with mulberry alkaloids meal

We observed that mulberry latex meal (0.1 g latex mixed with 10 g normal feed per day) caused diarrhea in chickens (data not shown). To investigate which ingredient of latex is the cause of chicken diarrhea, two groups of chickens were separately fed with the latex alkaloids and proteins meal. 0.1 g latex contains about the equivalent alkaloids in a 5% mulberry daily meal.

In the alkaloid group with normal feed the chicken's watery unformed feces rate was 5.2% daily and with alkaloids meal the rate increased significantly to 18.2% (P < 0.001). In the protein group, the watery unformed feces rate was 4.6% with normal feed and 5.3% with protein meal and did not show any significant difference (Table 1).

Alkaloids content of mulberry decreased after removing the latex and fermentation with *L. acidophilus*.

Mulberry Husang 32 is widely cultivated in China. DNJ is one of the three alkaloids in mulberry latex. Here, DNJ content was taken as the index of alkaloids. It showed that the DNJ content of untreated Husang 32 was 0.19% (dry weight). After washing away the latex the DNJ content decreased to 0.15%, while after a fermentation with *L. acidophilus*, the content was 0.17%. Washing latex and thereafter a fermentation resulted in the DNJ content decreased to 12% (Table 2).

The mulberry inhibition on starch hydrolysis decreased after removing the latex and fermentation with *L. acidophilus*.  $\alpha$ -glucosidase is required for starch hydrolysis *in vivo*, while  $\alpha$ -glucosidase inhibitors inhibit the hydrolysis. The Husang 32 extract showed 35% inhibition on starch hydrolysis, while that with latex washed away showed 17.2% inhibition. It indicated that the latex contributed a major inhibition on carbohydrate hydrolysis. With latex

**Table 2:** DNJ content of mulberry Husang 32 (dry weight).

Type of mulberry	DNJ content (%)
Natural mulberry	0.19
Fermented mulberry	0.17***
Washed mulberry	0.15***
Washed and fermented mulberry	0.12***

\*\*\*P < 0.001.

**Table 3:** The mulberry inhibition on starch hydrolysis.

Type of mulberry	Inhibition rate (%)
Natural mulberry	35.0
Fermented mulberry	25.7***
Washed mulberry	17.2***
Washed and fermented mulberry	14.6***

\*\*\*P < 0.001.

**Table 4:** Effects of mulberry meal on piglets weight gain.

Type of mulberry	Weight gain (kg)
Natural mulberry feed (A)	5.91
Washed mulberry feed (B)	6.85*
Washed and fermented mulberry feed (C)	7.68**

\*\*P < 0.01; \*P < 0.05.



**Figure 1:** The defecation of piglets with ordinary mulberry meal.

washed and fermentation, the inhibition decreased further to 14.6% (Table 3).

The mulberry edibility improved after removing the latex and a mild fermentation with *L. acidophilus*. Piglets were chosen for testing mulberry's edibility. The piglets eating habits and defecation were observed. When 10% mulberry

was mixed into piglet diets (group A), the piglets grew relatively slow in weight gain (Table 4) and showed diarrhea as compared with the normal feed. After reducing the alkaloids content of mulberry (group B), the piglets showed significantly a reduced defecation (Figures 1 and 2), increasing weight gain and improved appetite (Table 4).



**Figure 2:** The defecation of piglets with treated mulberry (washed away of latex) meal.

For the mulberry meals, the piglets exhibited better appetite to the fermented mulberry with higher weight gain (Table 4). The total protein content in mulberry Husang 32 is 30.1% (dry weight). After removing the latex, the content was 27.4%, and later became 28.0% with fermentation.

## DISCUSSION

Mulberry latex has high content of sugar-mimic alkaloids. These alkaloids inhibit carbohydrates digested into glucose, which results in the accumulation of hyperosmotic material and water exudation within the intestine and causes watery feces forming diarrhea of animals.

The fermentation with *L. acidophilus* improved the favor of mulberry. Our data indicated that it also reduced the alkaloids content. It is probably due to the acid produced by *L. acidophilus* depleting the alkaloids.

Although the excessive alkaloids content of mulberry is a limitation for its application as animal feed, the alkaloids collected from the washed away latex can be used in functional foods for blood glucose control or weight loss, which reflects another role of mulberry. It showed that type II diabetes blood glucose level can be effectively reduced by 20 mg DNJ per day (Ok et al., 2012).

Mulberry varieties show rich diversity in alkaloids content (Konno et al., 2006). The varieties with relatively low alkaloids and high protein content can be selected to accommodate feed industry.

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