Studies on *Medicago lupulina* saponins. 3. Effect of *M. lupulina* saponins on the growth and feed utilization by mice

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Abstract

The effect of *M. lupulina* saponins on the growth of mice and feed utilization was investigated. The nutritive value of diets containing different amounts of *M. lupulina* meal or saponins was evaluated using a diet containing clover meal as the control. Feeding experiments revealed that *M. lupulina* meal in concentrations higher than 5% in the diet caused significant growth depression in mice. The diet containing 30% *M. lupulina* meal was toxic to mice. It was shown that the fraction of saponins which contains medicagenic acid glycosides was responsible for the antinutritional properties of *M. lupulina* meal. The diet containing more than 0.1% of toxic *M. lupulina* saponins caused significant growth depression in experimental mice. A high dose of those saponins in the diet (0.5%), had a large inhibitory effect on mouse growth and feed utilization.

Key words: *Medicago lupulina*, saponins, antinutritional effect

INTRODUCTION

In our previous paper (Górski et al. 1984) we found that *M. lupulina* saponins contained two chemically and biologically different fractions. The first fraction containing medicagenic acid glycosides was highly toxic to fish and showed haemolytic and fungistatic properties. The second which contains soyasapogenol glycosides was not toxic to fish and did not show such activities.

Medicagenic acid glycosides isolated from alfalfa (*Medicago media* Pers.) are known to cause growth depression when fed to monogastric

animals. That effect, called antinutritional, was closely studied in alfalfa because alfalfa meal and leaf protein concentrate (LPC) are used as components in the diet of monogastric animals — chicks, broilers, swine (Chee et al. 1977).

The saponins from *M. lupulina* have not been investigated so far, although *M. lupulina* is widely grown and used as a feed. Hence the aim of our present study was to analyse the antinutritional properties of *M. lupulina* saponins.

MATERIAL AND METHODS

Plant material. Field-grown *M. lupulina* cv. Renata was used. The tops were cut at the stage of flowering, oven-dried at 60°C and ground. Alfalfa meal (*Medicago media* Pers.) cv. Kleszczewska and white clover meal (*Trifolium repens*) cv. Radzikowska was obtained in the same way.

Animals. Weanling white mice of both sexes weighing initially 13 ± 0.5 g (at 21-days old) were used. Mice were randomly divided into 10 experimental groups, 5 males and 5 females per group. The mice were kept in wire bottomed cages and weighed every day.

Diets. Three feeding experiments were conducted. In all experiments mice were fed ad libitum diets in the form of cakes. The first (diets No.: 1, 2 and 3) and third experiments (diets No.: 1, 6 and 7) were 16-days long. The second experiment was 20 days long and the mice were fed diets No.: 1, 3, 4, 5 and 6. The composition of the diets is presented in Table 1.

Saponin isolation and chemical analysis. The saponin content was measured using the *Trichoderma viride* biotest (Jurzysta 1979), the

Table 1
Composition of diets fed to mice (% in dry matter)

Component	Diet number							
	1	2	3	4	5	6	7	8
Wheat flower	60	60	60	60	60	60	60	60
Casein	5	5	5	5	5	5	5	5
<i>M. lupulina</i> meal	0	0	30	5	10	15	0	0
<i>M. media</i> meal	0	30	0	0	0	0	0	0
Clover meal	30	0	0	25	20	15	30	30
Saponins add.	0	0	0	0	0	0	0.07	0.4
Vitamin+salt*	5	5	5	5	5	5	5	5
Saponin content in the diet	0	0.27	0.54	0.09	0.18	0.27	0.07	0.40

* Salt (4%) and vitamin (1%) mixture as recommended by NRC USA (1976) and AOAC (1975) respectively — reported by Rakowska et al. (1978).

protein content was analysed by DBC method using a Pro-Meter MK II 14900 apparatus. Medicagenic acid glycosides were obtained from *M. lupulina* tops with cholesterol precipitation as previously reported (Górski et al. 1984).

RESULTS AND DISCUSSION

In the first feeding trial the nutritive value of *M. lupulina* meal was analysed and compared with that of alfalfa (*Medicago media* Pers.) and white clover (*Trifolium repens*) meals. *M. lupulina* meal contained 1.8% toxic saponins in dry matter and alfalfa meal contained 0.9% of these saponins in D M. Clover meal was chosen as the control because it contained only nontoxic saponins of soyasapogenols (Walter et al. 1955). The composition of other nutritive components in clover and alfalfa meals was similar (Ziołocka and Kuźdowicz 1981). We found the protein content of *M. lupulina*, alfalfa and clover meals to be 15.8, 15 and 15% of DM, respectively.

The growth rates of mice fed the diets containing these three meals are presented in Fig. 1. No weight gain in mice was observed on the diet containing *M. lupulina* meal and on the 15th day the death of one mouse was observed. A moderate weight gain in mice compared to the control was obtained with the diet (No. 2) containing alfalfa meal. The saponin content in diet No. 2 was two times lower than in diet No. 3 containing *M. lupulina* meal.

In the second feeding trial the diets containing 0.5, 10, 15 and 30%

Fig. 1. Body weight gain of mice fed diets containing 30% of clover meal (1), 30% of *M. media* meal (2) and 30% of *M. lupulina* meal (3). Final gains with asterisk were significantly different from control (1) by the Student's t-test at 5% level

M. lupulina meal were fed to the mice. The results of that experiment are presented in Fig. 2. It was found that the diet containing 5% *M. lupulina* meal was not significantly inferior to the control one. But higher concentrations of *M. lupulina* meal in the diet lowered the weight-gains of the mice.

Fig. 2. Body weight gain of mice fed diets containing the following amounts of *M. lupulina* meal: 1 — control, 4—5%, 5—10%, 6—15%, 3—30%. For details see Table 1. * — final gain was significantly different from control, $p < 0.05$

Distinct growth depression was observed in mice fed the diet containing 15% *M. lupulina* meal. In the second experiment, the diet containing 30% *M. lupulina* meal was more toxic than in the first experiment, because the death of one mouse was observed on the 6th day of trial.

The effect of *M. lupulina* concentration in the diet on the growth-rate of mice is presented in Fig. 3. It was found that body weight gain of mice fed the diet containing 10% of *M. lupulina* meal was 27% ($p < 0.05$) and the diet containing 15% meal, the weight gain was 6% ($p < 0.01$). A body weight gain of 38% was obtained for the control mice.

The third experiment was conducted to confirm the antinutritional role of saponin in the diet. In the experiment, mice were fed diets (No. 7 and 8) containing clover meal (control) supplemented with saponins (medicagenic acid glycosides) isolated from *M. lupulina* tops.

The results presented in Fig. 4 show that the diet containing only 0.07% toxic saponins is as good as the control one. However, with the

Fig. 3. The effect of the concentration of *M. lupulina* meal in the diet on the growth rate of mice

Fig. 4. Body weight gain of mice fed control diet (1) and control diets with addition of isolated saponins: 7 — 0.07%, 8 — 0.4%. * — final gain was significantly different from control, $p < 0.05$

concentration of 0.4% saponins, the diet caused significant growth depression in mice ($p < 0.05$).

In all experiments we observed that low gain in mice was correlated with a low consumption of the diet (Table 2). The poor feed utilisation may be due to the palatability of the saponins contained in the diet. This suggestion by Cheecke et. al. (1977), Kendall and Leath (1976) seems to be probable on the grounds of our findings, although the mechanism by which saponins cause growth depression has not yet been fully established.

Table 2

Body weight increase and feed consumption by mice fed diets containing different amount of saponins

Item	<i>M. lupulina</i> meal concentration in the diet, %				
	0	5	10	15	30
Saponin content in the diet, %	0	0.09	0.18	0.27	0.54
Daily average diet consumption, g/mouse	0.42	0.40	0.38	0.36	0.20*
Daily average increase of body weight, g/mouse	0.24	0.22	0.17	0.04	-0.48*

* Feeding trial with that diet was finished on the 6th day. Other diets were fed to mice for 20 days.

In our experiments we found that *M. lupulina* meal showed strong antinutritional activities and that saponins were responsible for this effect. Similar attributes of alfalfa saponins were described earlier (Chee c k e 1971).

The strong antinutritional effect of *M. lupulina* meal was partially due to the high concentrations of saponins in the meal (1.8%). Alfalfa meal containing two times less saponins (0.9%) was less toxic to mice.

On the other hand, *M. lupulina* saponins showed higher fungistatic and haemolytic properties (G ó r s k i et al. 1984). Now it seems that *M. lupulina* saponins are also more toxic than alfalfa saponins when fed to monogastric animals.

High concentrations of toxic saponins in the tops of *M. lupulina* and the strong antinutritional properties of the saponins make *M. lupulina* meal unsuitable as feed for monogastric animals.

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Badania nad saponinami Medicago lupulina. 3. Wpływ saponin M. lupulina na wzrost i wykorzystanie paszy przez myszy

Streszczenie

Badano wpływ saponin lucerny chmielowej na wzrost i wykorzystanie paszy przez myszy. Mysiom podawano paszę o zróżnicowanej zawartości mączki z lucerny, paszę kontrolną zawierającą mączkę z koniczyny oraz paszę kontrolną z dodatkiem wyizolowanych z lucerny saponin. Na podstawie doświadczeń żywieniowych wykazano, że pasza zawierająca więcej niż 5% mączki lucernianej powoduje znaczące zmniejszenie przyrostu ciężaru myszy. Pasza zawierająca 30% mączki z lucerny była dla nich toksyczna. Stwierdzono, że antyżywniowe działanie mączki z lucerny chmielowej wywołane jest przez saponiny — glikozydy kwasu medikagenowego. Wykazano również, że pasza zawierająca więcej niż 0.1% toksycznych saponin powoduje znaczące zahamowanie wzrostu myszy oraz, że duża zawartość tych saponin w paszy (0.5%) powoduje silne zahamowanie wzrostu oraz zmniejszenie pobierania paszy przez myszy.