Effect of Dietary Saponin on the Performance and Plasma Cholesterol Level of Chicks and the Alleviation of Saponin Toxicity by Cholesterol Supplementation

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Abstract Saponin toxicity to young chicks and its alleviation by cholesterol were investigated. Dietary saponin depressed chick growth and reduced feed consumption, and the depression rate was increased as the saponin level increased. The growth depression caused by 0.4% and 0.8% saponin supplementation to the soybean protein diet was completely counteracted by 0.4% and 0.8% cholesterol supplementation, respectively. The diet containing alfalfa leaf protein as the sole protein source contained 0.6% saponin and depressed chick growth, and it was alleviated by 0.6% cholesterol supplementation. When the LPC diet was added with 0.4% saponin, chick growth was more depressed, but was reversed by 1.0% cholesterol supplementation. Plasma cholesterol level of the chicks fed the soybean protein diet was about 200 mg/dl, and the level was decreased by feeding the alfalfa LPC diet or by addition of saponin to the diets. The decreased level was reversed to the normal level by supplementation of the adequate amount of cholesterol. It is concluded that a diet containing alfalfa LPC depressed chick growth because of the presence of saponin. Such toxic effect of saponin can be alleviated by cholesterol supplementation at the same level as that of the saponin present in the diet.

Key words: alfalfa LPC, saponin, cholesterol, plasma cholesterol, soybean protein, chick

Tasaki et al.1) and Terapunthawat and Tasaki2-7) have extensively studied the nutritive value of alfalfa leaf protein concentrate (LPC) to young chicks. They demonstrated that amino acid composition of the LPC was not so much different from that of the soybean protein (SBP), however, digestibility of amino acids, particulary of methionine was very low compared with that of the SBP3,4). Further, ME value of the LPC for young chicks was quite low5). Then, they fed chicks the LPC diet supplemented with either deficient amino acids to meet the requirements or corn oil to increase ME value of the diet, and they found that the nutritive value of the LPC was still inferior to that of the SBP6).

Kodoras et al.8) demonstrated that dehydrated or sun-cured alfalfa meal showed a typical growth depression in chicks when the alfalfa meal was incorporated in their rations at a 20% level. They further indicated that the factor responsible for depressing chick growth was more highly concentrated in alfalfa leaves than in the stems9). Peterson10) demonstrated that a hot water extract of alfalfa meal depressed the growth


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of chicks. This extract had a hemolytic property which was destroyed by boiling the extract with cholesterol. Then he suggested that the material involved in the hemolytic property might be saponin.

Hegsted and Linkswiler\textsuperscript{11}) compared the protein quality of the LPC obtained from high and low saponin alfalfa. The results indicated that the low saponin LPC had a higher nutritive value in rats than the high saponin LPC. Amenuddin \textit{et al.}\textsuperscript{12}) reported that excellent growth and feed efficiency were recorded even when 40\% of low saponin LPC was incorporated in broiler diets. Terapuntuwat and Tasaki\textsuperscript{7}) also reported that 40\% of soybean protein in chick diets would be replaced with alfalfa LPC on nitrogen basis, if mineral composition of the diet was adjusted to the requirement.

Heywang and Bird\textsuperscript{13}) demonstrated that the saponin extracted from alfalfa inhibited the growth, feed consumption and feed efficiency in chicks, and Cowlishaw \textit{et al.}\textsuperscript{14}) also demonstrated that alfalfa LPC was possibly due to the saponin contained in alfalfa, and this effect was alleviated by cholesterol supplementation. This phenomenon was also confirmed by Peterson\textsuperscript{10}) and Ueda and Ohshima\textsuperscript{15}).

The objective of the present experiment was to confirm the deteriorative effect of saponin and alleviatory effect of cholesterol, and also to investigate the quantitative relationship between saponin and cholesterol.

\textbf{Materials and Methods}

Alfalfa leaf protein concentrate (LPC) used in this experiment was prepared at the Nagoya University Farm by heating alfalfa juice at 65\(^\circ\)C. Soybean protein (SBP) was purchased from the private company.

The experiment consisted of 3 trials. In Experiment 1, effects on chick performance of saponin supplementation to a SBP diet (CP 18\%, ME 12 kJ/g) and of cholesterol supplementation to a LPC diet (CP 18\%, ME 12 kJ/g) were determined. The saponin used in all experiments was tea saponin. The SBP diet was formulated using 21.07\% SBP, 0.21\% DL-methionine, 51.07\% cornstarch, 1.00\% corn oil, 22.64\% cellulose, 3.78\% mineral mixture\textsuperscript{7}), 0.08\% vitamin mixture\textsuperscript{16}) and 0.15\% choline chloride. The LPC diet contained 40.42\% alfalfa LPC, 0.01\% L-arginine, 0.18\% DL-methionine, 51.61\% cornstarch, 1.00\% corn oil, 3.06\% cellulose, 3.49\% mineral mixture\textsuperscript{7}), 0.08\% vitamin mixture\textsuperscript{16}) and 0.15\% choline chloride. At the expense of cellulose, 1\% saponin and 1\% cholesterol were added to the SBP and LPC diets, respectively.

Experiment 2 was conducted to investigate the deteriorative effect of saponin supplemented to the SBP diet on its nutritive value and the alleviation of deteriorated nutritive value by supplementation with cholesterol. The basal SBP diet (CP 18\%, ME 12 kJ/g) was formulated with 21.04\% SBP, 0.12\% DL-methionine, 52.39\% cornstarch, 1.00\% corn oil, 19.05\% cellulose, 6.17\% mineral mixture\textsuperscript{17}), 0.08\% vitamin mixture\textsuperscript{16}) and 0.15\% choline chloride. This diet contained 0.08\% soya
saponin. At the expense of cellulose, saponin and/or cholesterol were added to the diet in various combinations. The levels of both saponin and cholesterol added were 0%, 0.4%, and 0.8%, and 9 combinations (saponin/cholesterol in %), i.e., 0/0, 0/0.4, 0/0.8, 0.4/0, 0.4/0.4, 0.4/0.8, 0.8/0, 0.8/0.4 and 0.8/0.8 were made. For example, 0.4/0.8 means that 0.4% saponin and 0.8% cholesterol were added to the diet at the expense of 1.2% cellulose.

Experiment 3 was planned to investigate the effect of saponin and/or cholesterol addition to the LPC diet. The basal LPC diet was formulated with 44.59% alfalfa LPC, 0.20% DL-methionine, 0.20% L-lysine, 37.42% cornstarch, 1.00% corn oil, 10.19% cellulose, 6.17% mineral mixture, 0.08% vitamin mixture and 0.15% choline chloride. In the same manner as Experiment 2, saponin and/or cholesterol were added to the basal LPC diet at the expense of cellulose. The combinations of saponin and cholesterol (saponin/cholesterol in %) were 0/0, 0/0.6, 0/1.2, 0.4/0, 0.4/0.4, 0.4/1.0 and 0.4/1.6, since the saponin content of the LPC diet was determined to be 0.6%. In addition to the LPC diets, the SBP diet which was composed of 21.30% SBP, 0.20% DL-methionine, 52.06% cornstarch, 1.00% corn oil, 19.04% cellulose, 6.17% mineral mixture, 0.08% vitamin mixture and 0.15% choline chloride, was used as a control.

In all experiments, single comb White Leghorn male chicks were used. Day-old chicks were placed in electrically heated battery brooders and given a commercial chick starter diet for 7 days. On day 8, the chicks were individually weighed after 3-hour fasting, and divided into 4 groups in Experiment 1, 9 groups in Experiment 2 and 8 groups in Experiment 3, of 5 birds each, so that the average initial body weight in each experiment was quite uniform, being 66, 71 and 76 g in Experiments 1, 2 and 3, respectively. The chicks were kept individually in metabolism cages. The ambient temperature was maintained at 29 ± 2°C and lighting was provided continuously throughout the experiment. The experimental diets and water were given ad libitum for 10 days from day 8 to day 18, and on the final day body weight was measured after 3-hour fasting. Then, blood samples were collected by heart puncture in Experiments 2 and 3, and liver weight was measured in Experiment 2. Plasma cholesterol was determined by the modified o-phthalaldehyde method, and saponin content by the method of Van Atta et al. Data were analyzed using the analysis of variance technique and multiple comparisons among treatment means were made by Duncan's new multiple range test.

Before starting the experiment, an effect of fasting time on plasma cholesterol level was examined. A group of 60 chicks were reared on a commercial chick diet, and they were fasted at 16 days of age. Blood samples were taken at 5-minute intervals from 0 to 4 hours and at 7, 10 and 24 hours after fasting, and cholesterol content of blood plasma was determined. As a result, no difference in plasma cholesterol was observed between any cases of fasting time, and the blood collection was done between 3 and 7 hours of fasting in Experiments 2 and 3.
Results

Chick performance in the Experiment 1 is shown in Table 1. Body weight gain and feed intake on the SBP diet were superior to those on the LPC diet, but gain/

Table 1. Effect of saponin and cholesterol on chick performance (Experiment 1)

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weight gain g/10 days</th>
<th>Feed intake g/10 days</th>
<th>Gain/feed</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>63.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>154.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.41&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>+1.0% saponin</td>
<td>14.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LPC</td>
<td>47.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>118.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.40&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>+1.0% cholesterol</td>
<td>74.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>147.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.50&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSR* (df=16)</td>
<td>6.7</td>
<td>15.6</td>
<td>0.04</td>
</tr>
</tbody>
</table>

* Least significant range (FEDERER<sup>20</sup>). Values with different superscript letters in the same column differ significantly at P<0.01.

*Fig. 1.* Effect of saponin (Sap) and cholesterol (Chol) supplementation to the soybean protein (SBP) diet on chick performance (Experiment 2). Vertical bars indicate least significant range (LSR) of means (df=36). Different letters indicate significant difference at P<0.01.
feed ratio was almost the same between both treatments. When 1% saponin was added to the SBP diet, all parameters declined remarkably, especially the reduction of body weight gain was quite large. On the contrary, addition of 1% cholesterol to the LPC diet increased body weight gain and feed intake. Especially, body weight gain was significantly larger than that on the SBP diet, and efficiency of feed utilization was remarkably improved. Then, effects of the supplementation with saponin and/or cholesterol to the SBP diet at various levels were compared in Experiment 2, and the results are shown in Figure 1.

When the basal SBP diet was added with 0.4% and 0.8% saponin, feed intake was reduced from 161 g to 139 g and 118 g, respectively, and body weight gain was also decreased from 68 g to 47 g and 21 g. The reduction rate was much larger in body weight gain than in feed intake, the former being 31% and 69% and the latter 13% and 27% in 0.4% and 0.8% saponin supplementation, respectively. Reflecting these, gain/feed ratio was significantly lower in 0.4% saponin supplementation, than in 0.8% and both values were significantly lower than that on the basal diet. When the basal diet was added with 0.4% or 0.8% cholesterol but not with saponin, no effect was found on chick performance.

When the 0.4% saponin diet was added with 0.4% or 0.8% cholesterol, the deteriorative effect of saponin on chick performance was completely reversed by cholesterol, and no significant difference was found between the levels of cholesterol. When the 0.8% saponin diet was added with 0.8% cholesterol, the deteriorative effect of saponin disappeared, however, when the addition of cholesterol was reduced to 0.4%, body weight gain and feed intake were not completely recovered to the basal level.

No difference in liver weight was found between any treatments, being 2.9–3.2 g/100 g body weight. Plasma cholesterol level of the chicks fed the basal diet was 202 mg/dl as shown in Figure 2, and the level was decreased to 151 and 155 mg/dl by addition of 0.4% and 0.8% saponin, respectively. When 0.8% cholesterol was added.

![Fig. 2. Effect of saponin (Sap) and cholesterol (Chol) supplementation to the soybean protein (SBP) diet on plasma cholesterol level in chicks (Experiment 2). Vertical bar indicates least significant range (LSR) of means (df=36). Different letters indicate significant difference at P<0.01.](image-url)
to the basal diet the plasma cholesterol level was significantly increased to 274 mg/dl, while the addition of 0.4% cholesterol did not increase the plasma cholesterol level. The addition of cholesterol to the 0.4% saponin diet at the levels of 0.4% and 0.8% significantly increased plasma cholesterol level to 246 mg/dl for the former and 273 mg/dl for the latter, and the former lever was higher than the level for the SBP diet with 0.4% cholesterol, the latter level was the same as the level in the SBP diet with 0.8% cholesterol. The addition of 0.4% cholesterol to the 0.8% saponin diet, plasma cholesterol level did not increase, however, the addition of 0.8% cholesterol significantly increased plasma cholesterol level to 226 mg/dl.

The results of Experiment 3 are illustrated in Figures 3 and 4. As shown in Figure 3, the LPC diet apparently depressed chick growth and reduced feed intake, since those values were lower than those on the SBP diet. Similar to the result of Experiment 1, gain/feed ratio was not different between the LPC and SBP diets.
When 0.4% saponin was added to the LPC diet, feed intake, body weight gain and gain/feed ratio were significantly decreased not only from the control values but also from the values on the LPC basal diet, being 80%, 67% and 84% those of the LPC basal diet, respectively. The addition of either 0.6% or 1.2% cholesterol to the LPC diet increased body weight gain and feed intake to the control levels. When 0.4% cholesterol was added to the 0.4% saponin diet, feed intake was almost recovered to that of the LPC basal diet, while body weight gain was still significantly lower than that of the LPC basal diet. The addition of 1% cholesterol to the 0.4% saponin diet improved the chick performance to the level of the control, however, no further improvement was found thereafter.

Plasma cholesterol level of the chicks fed the LPC basal diet was 160 mg/dl as shown in Figure 4, and this level was significantly lower than the control level. The addition of 0.6% cholesterol did not affect the plasma cholesterol level, however, the addition of 1.2% cholesterol significantly increased the plasma cholesterol to the control level. The addition of 0.4% saponin to the LPC diet significantly reduced the plasma cholesterol level, and by the addition of 0.4% cholesterol the plasma cholesterol level was recovered to the level of the LPC basal diet, but further improvement was not observed thereafter.

**Discussion**

HENWANG and BIRD\(^{13}\) fed diets containing 0 to 0.4% alfalfa saponin to chicks, and they found that the alfalfa saponin depressed growth, feed consumption and feed efficiency in chicks as the order of saponin levels. It was shown in the present experiments that the alfalfa saponin and tea saponin reduced chick growth, feed consumption and feed efficiency and the degree of toxicity became larger as the saponin level was increased. No ill-effect was detected in the SBP diet, since the diet contained only 0.08% saponin and the quantity of saponin was too small to show an ill-effect, if any. PETERSON\(^{21}\) demonstrated that quillaja saponin depressed chick growth, and MORGAN
Peterson demonstrated that the growth depression in chicks caused by a high level of alfalfa saponin in a diet was completely counteracted by the addition of cholesterol to the diet. In the present experiment the similar results were obtained when an adequate amount of cholesterol was supplemented to the diet containing saponin. In Experiment 2, the depression of both body weight gain and feed consumption caused by either 0.4% or 0.8% saponin supplementation was completely counteracted by the supplementation of 0.4% or 0.8% cholesterol, respectively, and 0.8% saponin could not completely counteracted by 0.4% cholesterol.

In Experiment 3, the LPC diet contained 0.6% saponin, and the diet added with 0.4% saponin contained 1.0% saponin. The deteriorative effect of the LPC diet was alleviated by the addition of 0.6% cholesterol, and that of the 0.4% saponin diet was alleviated by the addition of 1.0% cholesterol but not by the 0.4% cholesterol supplementation.

It is considered from these results that the amount of cholesterol to counteract the saponin toxicity should not be less than the same amount of saponin present in the diet, and an extra amount of cholesterol does not show any beneficial effect.

Chen et al. observed that cholesterol supplementation to a casein-dextrin diet tended to increase liver size in rats, however, such tendency was not found in the present experiment.

Wilcox and Galloway reported that dietary alfalfa saponin lowered serum cholesterol level in the cholesterol-fed rats. Morgan et al. also reported that both gypsophila saponin and digitonin lowered serum cholesterol level of rats. As shown in Figures 2 and 4 in the present experiments, plasma cholesterol level was lowered by saponin supplementation, and recovered by the addition of cholesterol, however, overdosage of cholesterol induced hypercholesterolemia.

Peterson demonstrated that supplementation with either cholesterol or diethylstilbestrol increased blood cholesterol level in chicks, however, the diethylstilbestrol treatment was ineffective as a means of preventing the growth depression brought about by a high alfalfa meal diet. Then, he suggested that counteraction of the alfalfa growth inhibitor, saponin, was not dependent upon an increase in the plasma cholesterol level, and that the alleviatory effect of cholesterol was due to the formation of an insoluble cholesterol-saponin compound (cholesterol saponide) in the digestive tract. The Peterson's suggestion that the increased plasma cholesterol level would not be responsible for alleviation of saponin toxicity was also confirmed by Morgan et al. and in the present experiments. Morgan et al. further pointed out that if the Peterson's suggestion that the formation of cholesterol saponide would be responsible for alleviation of saponin toxicity were true, it could also account for the hypocholesterolemic effect of dietary saponin. Indeed, they could not demonstrate the interference of cholesterol absorption by digitonin and also they could not find cholesterol-digitonin compound in the intestinal tract of chicks. They further observed that 3β-hydroxy-3β-cholestanol, a sterol fully precipitable with digitonin and differing only slightly in
its chemical structure from cholesterol, failed to reverse the digitonin-induced growth depression, whereas the growth depression induced by gypsophila saponin was almost completely reversed by cholesterol, even though it bound with this saponin to a small degree only. If an elevation of plasma cholesterol level reflects an increased cholesterol absorption, the addition of cholesterol at levels more than the adequate amount should increase plasma cholesterol as shown in Figure 2 in the present experiment, however, as shown in Figure 4 the addition of 1.6% cholesterol to the diet containing 1.0% saponin did not increase the plasma cholesterol level. The formation of cholesterol saponide might be one reason to alleviate saponin toxicity, but the reversal of saponin-induced growth depression by cholesterol would still appear to need further investigation as suggested by Morgan et al.\textsuperscript{22}

It could be concluded that a diet containing alfalfa LPC depresses chick growth because of the presence of saponin. Such toxic effect of saponin can be counteracted by cholesterol supplementation at the same level as that of the saponin present in the diet.

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\textbf{References}

鶏ヒナの発育および血中コレステロールに及ぼす飼料中サポニンの影響とコレステロールによるサポニンの毒性緩和効果

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鶏ヒナに対する飼料中サポニンの有害作用と、コレステロールによるその緩和効果について検討した。すなわち飼料中サポニンはその含量に応じてヒナの成長を阻害し、また飼料摂取量を低下させた。大豆蛋白を主成分とする飼料に 0.4% または 0.8% のサポニンを添加することにより生じたヒナの成長低下は、それぞれ 0.4% または 0.8% のコレステロールを飼料中に添加することにより完全に防止することができた。アルファルファ LPC を主成分とする飼料は 0.6% のサポニンを含有しており、これを給与すると大豆蛋白質飼料に比較してヒナの成長が減退したが、0.6% のコレステロールを添加すればそれれを防ぐことができた。LPC 飼料にサポニンを 0.4% 添加すると発育阻害の程度は高くなったが、これも 1.0% のコレステロール添加により防止できた。大豆蛋白飼料を給与したヒナの血漿中コレステロールは約 200 mg/dl であるが、これにサポニンを添加するか、または LPC 飼料を給与すると血漿中コレステロール濃度は低下した。しかし飼料中に適量のコレステロールを添加すると、血漿中コレステロールは通常レベルにまで回復した。以上の結果より、アルファルファ LPC はそれに含有されるサポニンの作用によりヒナの発育を抑制するが、サポニンの含量と同様のコレステロールを飼料中に添加すればサポニンの害作用を除去できることができ明らかになった。日畜会報, 57 (6): 524-533 1986