Research Notes

Liver Proteolytic Activity in Tannic Acid-Fed Birds

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ABSTRACT The influence of tannic acid in the rate of growth (BWG), feed intake, protein efficiency ratio, and liver proteolytic activities (cathepsin A and D) were measured in growing male chickens. These birds were fed ad libitum over a 15-d experiment on 20% protein standard diets containing heated soybean (control, C) as the main source of protein. Tannic acid (TA; 25 g/kg diet) was added to all diets, except the control. It has been found that in comparison to control-fed birds, TA-fed birds showed a significant reduction (P < 0.01) in BWG, protein efficiency ratio, and relative weight of liver, together with a significant increase (P < 0.01) in the activities of cathepsin A and D in liver. Addition of TA to the control diet had no significant effect on feed intake. The possible nature of these results is discussed.

(Key words: chicken, tannic acid, liver, cathepsin A, cathepsin D)


INTRODUCTION

Tannins are present in many products of vegetable origin that are used as human foods or animal feeds. Although tannins are chemically not well defined, they are usually divided into hydrolyzable and condensed tannins. Hydrolyzable tannins have a central carbohydrate core whose hydroxyl groups are esterified to phenolic carboxylic acids, such as gallic acid, ellagic acid, and hexahydroxydiphenic acid. Esters of the first two acids are referred to as gallotannins. Tannic acid (TA) is a well-known gallotannin and contains 8 to 10 mol of gallic acids/mol of glucose. These types of tannins are readily hydrolyzed by acids, alkali, or some enzymes. Upon hydrolysis, they yield glucose or some other polyhydroxy alcohol and gallic acid or some phenolic acids related to it (Freudenberg and Weinges, 1962; Haslam, 1966; Salunkhe et al., 1990; Jansman, 1993; Hagerman et al., 1997).

A number of adverse nutritional effects have been attributed to tannins. Studies have been conducted on the effects of tannins in feedstuffs on animal performance. Some of them have been carried out with tannins isolated from feedstuffs or with standard commercial tannins, such as TA, that were thought to be representative of tannins in many feedstuffs and with raw legumes containing different levels of tannins (Mitjavila et al., 1977; Wareham, 1993). It has been demonstrated that feeding growing animals diets containing these compounds brings about several undesirable physiological and biochemical effects. These effects are reflected by growth inhibition, negative nitrogen balances, reduced intestinal absorption of sugars and amino acids, reduced immune response, and increased liver and protein catabolism (Santidrián, 1981; Santidrián and Marzo, 1989). The liver is one of the body’s most sensitive organs to toxic factors or protein deficiency. Administration of diets to animals containing tannins should result in a greater decrease in liver protein than in other organs. Proteins seem to be influenced by protein catabolic processes (Cenarruzabetia et al., 1979; Lasheras et al., 1980), which have an obvious relevance in farm animal nutrition. Lysosomal proteolytic activity plays a decisive role in protein metabolism, with cathepsin A and D being the main enzymes involved in the catabolism process in the liver. For this reason, we have undertaken the present investigation to further elucidate catabolic action of tannins on chicken protein metabolism by studying the effect of the TA on the activities of cathepsin A and D in liver.

MATERIALS AND METHODS

One-day-old White Leghorn male chickens, weighing 40 to 50 g, were randomly assigned to two dietary groups of 10 birds each and were housed in battery brooders with raised floors. Groups were arranged as follows: one was fed a standard control diet, and the other was fed the same diet to which 25 g TA/kg diet was added. Both

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Abbreviation Key: BWG = rate of growth; TA = tannic acid.
water and feed were supplied ad libitum. The total protein content of each diet was 20%; the source was heated soybeans. Diets were prepared according to criteria previously reported (Marzo et al., 1989, 1991). Total caloric content was about 3,000 kcal/kg. Body weight changes were individually recorded every 3 d.

Within each experimental group, 10 chickens were killed by decapitation when they were 15 d of age. Immediately after bleeding, livers were removed and weighed. Liver protein content was assayed by the method of Lowry et al. (1951). Free liver cathepsin D activities were measured in control and TA-treated birds according to the method of Gianetto and DeDuve (1955). As in previous work (Santidrian et al., 1987), activities of cathepsin D are given in units per milligram of liver. One unit is defined as the amount of enzyme that catalyzes the liberation of 1 μmol of tyrosine (measured by ninhydrin reaction) from hemoglobin during incubation (30 min at 37°C).

Cathepsin A was assayed by the method of Iodice and Weinstock (1965); the steps involved were very similar to those mentioned for the cathepsin D, with the difference that N-CBZ-glutamyl-L-tyrosine was used as the substrate. TA (from Phaseolus helsvetica extracts), hemoglobin, and N-CBZ-glutamyl-L-tyrosine were purchased.1

### Statistical Analysis

For each parameter investigated, the data were statistically evaluated by one-way ANOVA. Comparisons between TA- and control-fed birds were made. Least-significant differences for P < 0.01 were calculated (Miller and Miller, 1993).

### RESULTS AND DISCUSSION

Results of the experiment are summarized in Table 1. As shown, the rate of growth (BWG) and relative weight of liver were significantly reduced (P < 0.01) in TA-fed chickens as compared with control-fed birds. Nevertheless, no significant changes were observed in feed intake, measured in grams of food consumed per bird over time. Protein efficiency ratios were diminished 20% (P < 0.01) in TA-fed birds. The reduced BWG and relative weight of other organs displayed by TA-fed chickens or those fed raw legumes correlate with previously reported data from this laboratory (Marzo et al., 1989, 1990, 1991) and others (Castañon and Marquardt, 1991). The toxicity of raw legume beans has been described in relation to tannin content. Although there is general agreement that feeding a diet containing these legumes as the sole source of protein exerts a toxic effect, it has been found that the toxic action of tannins may account, at least in part, for this growth impairment (Elkin et al., 1990).

Some studies have determined the effects of commercially available hydrolyzable tannins on chicks and rats. TA, when fed to different animal species, has been shown to affect the digestibility and absorption of nutrients but also to affect different internal organs (Chang and Fuller, 1964; Singleton, 1981). However, biochemical changes in the cathepsin activities in response to tannic acid content in diets have not been studied previously. Table 1 shows that, as compared with control birds, those fed the diet with TA exhibited a marked increase in the activities of cathepsin A and D in liver. This finding relates to the fact that feeding growing animals a diet containing raw legumes brings about an increase in protein catabolism.

Published evidence has shown increases in the activity of a number of hepatic amino acid-degrading enzymes in rats and chickens fed a raw faba bean diet (Cenarruzabeitia et al., 1979; Santidrian et al., 1981). Reports have also shown that TA or degradation products are absorbed from small intestine and cause toxic effects (Vohra et al., 1966; Kardivel et al., 1969; Karin et al., 1978). To explain, at least in part, the catabolic effect caused by tannins from raw legumes or by TA, the results of this experiment have shown relationship between TA and liver cathepsin A and D activities. Moreover, a relationship between the marked increase in liver proteolytic activity and the reduction in BWG may suggest that use of TA affects protein catabolism and the nutritive value of diets.

### REFERENCES


