



Journal of Biological Sciences

ISSN 1727-3048

science
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Condensed Tannin Contents of Some Legume Seeds Used in Fish Nutrition

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Abstract: The aim of this experiment was to determine the condensed tannin contents (CT) of legume seeds used in fish nutrition as alternative to fish meal. The CT contents of legume seeds ranged from 6.03 to 28.41 g kg⁻¹ DM. The CT contents of Tuntal Sırık and Barbunya were significantly ($p < 0.001$) higher than those of Bakla, Dolar Badem, Culban and Soya. Legume seeds studied in this experiment had the considerable amount of CT which can be detrimental effect on the nutritive value for fish. This experiment also clearly showed that there is positive correlation between color and CT of legume seed. The darker the seed color is the higher the CT content legume seed has. Therefore legume seed which had a white color are preferable for fish to decrease the possible detrimental effect of CT on the growth of fish. This information is also valuable to plant breeders to produce legume seed with low CT content.

Key words: Legume seeds, bean, condensed tannin

INTRODUCTION

Growth of aquaculture industry has increased fish meal demand, causing uncertainty in its availability (Davies *et al.*, 2004). Thus research efforts are continuing in search of cheap and alternative protein sources to minimize feed expenditure, especially cost of dietary protein. The utility of plant protein sources to exclusively or partly replace the fish meal protein is being investigated in many countries (Krishnankutty, 2005). Legume seeds are protein-rich ingredients incorporated in fish diets as alternatives to fish meal in various countries. However this often results in decreased apparent digestibility and impaired fish performance due to antinutritive factors such as protease inhibitors, tannin, lectins and some other unidentified compounds in legume protein sources.

Although recently a lot of works have been carried out to determine the antinutritive factors of legume seeds the CT contents of legume seeds were ignored since the CT of legume seeds was possibly thought to be lower than the level which has a deleterious effect on the growth of fish. However preliminary experiment showed that some of legume seed had the high CT content which can be deleterious effect on growth of fish.

The aim of this experiment was to determine the CT contents of legume seeds used in fish nutrition as alternative to fish meal.

MATERIALS AND METHODS

Legume seeds were obtained from local market and ground to pass through 1 mm sieves. The CT content of legume seeds was determined according to the butanol-HCl methods described by Makkar *et al.* (1995). Ground seed samples (0.01 g) were weighed in triplicate tubes and 6 mL butanol-HCl (95:5) reagent added. The tubes were then placed into boiling water (100°C) and heated for 1 h after which they were removed, cooled and centrifuged at 3000 X g for 10 min. The supernatant was decanted into vials and absorbance read at 550 nm using a CE 2030 single beam spectrophotometer (Cecil Instruments, England). Blank samples containing the reagent were only included in the measurements.

Data on CT contents was subjected to the one way of ANOVA using GLM of Statistica for windows (1993). Significance between individual means was identified using the Tukey's multiple range test (Efe *et al.*, 2000). Mean differences were considered significant at $p < 0.05$. Standard errors of means were calculated from the residual mean square in the analysis of variance.

RESULTS

The CT contents and color of legume seed hulls are given in Table 1. There are significant ($p < 0.001$) variation among legume seeds in terms of CT and hulls. The CT contents of legume seeds ranged from 6.03 to 28.41 g kg⁻¹

Table 1: Condensed tannin and color of some legume seed

Legume species	Variety (Local name)	Color of seed hull	Condensed tannin
<i>Phaseolus vulgaris</i>	Tıntal sırık	Brown	28.41 ^c
<i>Phaseolus vulgaris</i>	Barbunya	Red	26.79 ^c
<i>Vicia sativa</i> L.	Adi fig	Dark brown	23.33 ^{bc}
<i>Vicia faba</i> L.	Bakla	Light Brown	10.89 ^a
<i>Phaseolus vulgaris</i>	American badem	Brown	23.25 ^{bc}
<i>Phaseolus vulgaris</i>	Ayşe kadın badem	Black	23.80 ^{bc}
<i>Phaseolus vulgaris</i>	Dolar badem	White	6.03 ^a
<i>Phaseolus vulgaris</i>	Teymen badem	Brown	24.10 ^{bc}
<i>Vicia pregrina</i> L.	Culban	Brown	14.15 ^{ab}
<i>Glycina max</i> L.	Soya	White	7.19 ^a
SEM			2.393
Sig.			***

Column means with common superscript do not differ ($p > 0.05$), SEM: Standard Error of Mean, Sig: Significance level, *** $p < 0.001$

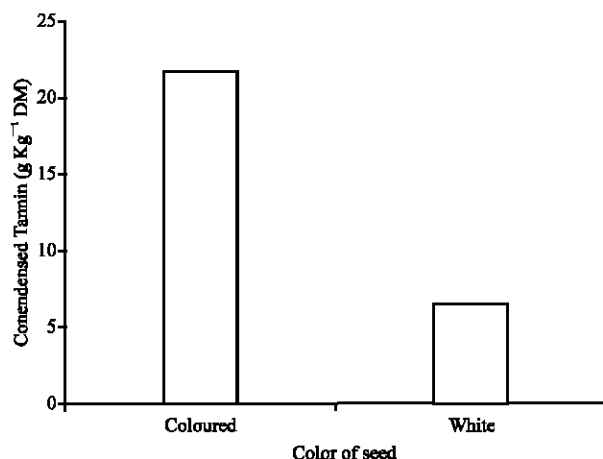


Fig. 1: The average condensed tannin content of coloured and white legume seed

DM. The CT contents of Tıntal Sırık and Barbunya were significantly ($p < 0.001$) higher than those of Bakla, Dolar Badem, Culban and Soya.

As can be shown from Table 1 there are also significant differences in color of hull of legume seeds. The color of seed hulls ranged from white to black. The legume seed with coloured hulls had ($p < 0.001$) significantly higher CT than those of legume seeds with white hulls.

The average CT contents of legume seed are given in Fig. 1. The CT content of legume seeds with coloured hulls was 3-4 times higher than those of legume seeds with white hulls.

DISCUSSION

Legume seeds show a very large variation in color and CT contents. Tannins are secondary compounds of various chemical structures widely occurring in plant kingdom and are generally divided into hydrolysable and condensed tannin (Francis *et al.*, 2001). The CT is located mainly in the seed hulls and plays an important role in the

defense system of seeds that are exposed to oxidative damage by many environmental factors such as light, oxygen, free radicals and metal ions (Trosynska and Ciska, 2002). There are variations in the content of CT in legumes depending on the color of seed hulls (Elias *et al.*, 1979). The white varieties of legume contain usually tannins in lower concentrations than red, black or bronze seed hulls. In this experiment it was found that the legume seeds with white hulls had a lower CT content than those with coloured hulls. This results in agreement with finding of Elias *et al.* (1979).

On the hand antinutritional effects of CT include interference with the digestive processes either by binding the enzymes or binding to feed components like proteins or minerals (Liener, 1989). Tannin also reduces the absorption of Vitamin B12. The CT is reported to be responsible for growth depression, low protein digestibility, decreased amino acid availability and increased fecal nitrogen (Durigan *et al.*, 1987; Elias, 1979; Reddy *et al.*, 1985). In this experiment it was shown that legume seeds with coloured hull had high CT contents which can be detrimental to fish growth. However some experiments clearly showed that there were considerable differences among fish species in terms of the tolerance level of CT. Common carp has been shown to able to tolerate 2% addition of condensed tannin without any effect on growth whereas CT present in copra at a level of approximately 2.4% could have been the cause of growth depression in tilapia and rohu fingerlings even at such low levels of inclusion as 25 or 20% (Jakson *et al.*, 1982; Mukhopadhyay and Ray, 1999). These differences probably indicate the differences in tolerances of different fish species and differences in the structure of the tannins or their interactions with other components in diets (Francis, 2001). Therefore detannification methods can be suggested to decrease or eliminate the negative effect of CT in legume seeds with coloured hulls on fish nutrition. However the *in vivo* studies is required to determine the CT content of legume seeds with coloured hulls before large application of detannification methods. Recently

there are some widely used detannification methods such as de-hulling, autoclaving and alkali treatment (Griffiths, 1991).

CONCLUSIONS

Legume seeds studied in this experiment had considerable amount of CT which can be detrimental effect on the nutritive value for fish. This experiment also clearly showed that there is positive correlation between color and CT of legume seed. The darker the seed color is the higher CT content the legume seed has. Therefore legume seed which had white hulls are preferable for fish diets to decrease the detrimental effect of CT on the growth of fish. This information is also valuable to plant breeders to produce legume seed with low CT content.

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