THE ISOLATION AND CHARACTERIZATION OF AN ANTI NUTRITIONAL FACTOR IN FABA BEANS (VICIA FABA L. VAR. MINOR)

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Arthur Thomas Ward

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ABSTRACT

Various methods of heat treatment of faba beans were compared to establish their effectiveness in promoting improved chick growth. Microwave treatment was the least effective and autoclaving (121° 30 minutes) and extruding (152°) were the most effective of the various processing methods. Extruding increased chick weight gain 16% and feed:gain ratio 18% while autoclaving improved chick weight gain 17% and feed:gain ratio 10%. The moisture content of the faba beans was critical with regard to the response of chicks fed microwave irradiated as compared with raw faba beans. Trypsin inhibitors, hemagglutinin, other proteins and vicine were determined not to be responsible for the poor utilization of faba beans. The effects of heat treatment on chick response as indicated by body weight gain and efficiency of feed utilization were associated primarily with heat treatment of the protein fraction as compared to the starch fraction. Gelatinization of the starch had no effect on chick growth.

Comparing the hull and protein fractions demonstrated that the hull contained a potent inhibiting compound responsible for at least one-half of the growth inhibition of faba

beans. Chick weight gain and feed:gain ratio were markedly improved when birds were fed autoclaved as compared to raw faba beans and when the hull content of the diet was decreased. The maximum improvement due to heating a diet (20% hulls added) was 31% and 25% for weight gain and feed:gain ratio respectively. Decreasing the hull content in the diet from 20 to 1% improved weight gain and feed:gain ratio 20 and 33% respectively.

Water extraction and heating of the hull similarily improved chick growth response and nutrient retention. The dried extract upon being fed to chicks reduced weight gain 80% and increased the feed:gain ratio 35%. Autoclaving the extract before being added to the diet resulted in weight gains and feed:gain ratios equal to the control diets.

The inhibitor was further isolated by extracting the water extract with 90:10 acetone:water. This concentrated extract was dried, redissolved and applied to a Sephadex LH-20 column. Elution, firstly with 95% ethanol, then with 50:50 acetone:water yielded two distinct peaks. The first peak contained low molecular weight polyphenolic compounds while the second peak contained pure condensed tannins. Upon feeding these fractions to chicks it was found that most of the growth inhibitor was associated with the original water extract, the 90:10 acetone:water extract and the tannin peak obtained from the Sephadex LH-20 column. The first peak obtained from the Sephadex LH-20 column decreased feed intake due to its

astringent taste. Consequently weight gain was decreased but feed:gain ratio was the same as the control diets.

Chemical characterization of the two peaks from the Sephadex LH-20 column showed that they were different compounds with different chemical and physical properties. Using two dimensional thin layer chromatography, the peaks were separated into their basic components. Preliminary studies on the nature and chemical structure of the tannins indicated they were condensed tannins as compared to hydrolyzable tannins and that two of the subunits were delphinidin and cyanidin.

The growing conditions, storage conditions and age of the bean were shown to effect tannin levels. The average decrease in tannin levels over two years in Canadian grown beans was 37%. The maximum decrease of the varieties tested occurred in the cultivar Hertz-Freya which decreased 46% over two years. Local cultivars had quite similar levels of tannins being approximately 4.0% of the hull. European varieties varied from 0 to 6% of the hull. The varieties Kodrim, Fidrim and Triple White from Holland did not contain any measureable tannins.

INTRODUCTION

Faba beans are a high producing, high protein legume crop. Although they seem to have good protein quantity and quality, they do not support chick growth as would be expected. The poor growth response is attributed partially to the presence of growth inhibitors in the bean.

The purposes of this study were:

1. To determine the effect of different heat treatments on the nutritional quality of faba beans. This included;

    a) the effect of various methods of heating the bean, including steam pelleting, extruding, autoclaving and microwave irradiation.

    b) the effect moisture has on the effectiveness of some of the heat treatments.

    c) the length of time needed to get maximum chick growth with the best heat treatment process.

2. To determine in what portion of the bean the major growth inhibitor was associated. This included isolating the bean into its basic components, that is, protein, starch and hull and comparing the effect on chick growth when these components are added to diets either raw or heat treated.

3. To isolate the growth inhibitor from the faba bean fraction having the high inhibiting activity. This included extracting the component and concentrating it, then feeding it to chicks to determine its effects on growth.

4. To identify the inhibiting component. This included, separating the pure component by chemical means and running a number of chemical tests to determine the subcomponent structure.

5. To determine what effect this component has on a growing chick. This included studies on protein and amino acid retention of chicks fed beans or fractions thereof containing either the raw or heat treated inhibitor.

LITERATURE REVIEW

Faba Bean Gross Composition

The protein content of faba beans (N x 6.25) varies from approximately 25 - 35% on a dry matter basis depending on the growing conditions, location and variety of the bean (6, 15, 18, 49). The embryo and cotyledon represent approximately 86% of the bean with the hull representing 11 to 14%. The hull contains 45% crude fibre and 6% protein (12, 44, 49). The carbohydrate content of faba beans varies from 51 to 66% with 28 to 42% starch (6, 12, 73). The hull contains approximately 79% carbohydrate mostly as structural polysaccharides. Prichard (54) reported that of 8 varieties of field beans tested, winter-sown beans contained 46 to 48% available and 19 to 20% unavailable carbohydrate, while spring-sown beans contained 30 to 42% available and 22 to 37% unavailable carbohydrate on a dry matter basis. The available carbohydrate consisted of dextrins, water-soluble and insoluble starches and ethanol soluble sugars, while the unavailable carbohydrate fraction contained lignin, cellulose, hemicellulose and water-soluble polysaccharides. Small amounts of glucose containing polymers soluble in dilute acid are present in the cotyledon but

increase to 3.6% in the hull (12).

Comparing field bean (Vicia faba L.) meal as a protein source for colostomized hens Waring (72) reported true digestibility values of crude protein of field beans to be 83% and the metabolizable energy (M.E.) content to be 2910 kcal per kg. This compares with digestibility values of 89 and 69% for fish meal and meat-and bone meal and M.E. values of 2645 kcal per kg. and 1988 kcal per kg. respectively. Comparing eleven samples of field beans (Throws M.S.) representing different generations, locations and soil types Edwards and Duthie (19) reported a mean "classical M.E. value" of 2.40 ± 0.09 kcal per gram and a mean nitrogen corrected value of 2.26 ± 0.1 kcal per gram. Comparing winter sown to the above spring sown samples Edwards and Duthie obtained M.E. values for six samples of winter sown Throws M.S. of 2.42 ± 0.20 kcal per gram for classical mean and 2.33 kcal per gram for nitrogen corrected (21). Carpenter and Johnson (11), based on a single sample of Throws M.S. beans harvested in 1967 reported a M.E. value of 2.52 kcal per gram, thus indicating that growth and harvest conditions influence metabolizable energy values.

Plant proteins in general are deficient in one or more amino acids. Cereals are mainly deficient in lysine while legumes and leaf proteins are deficient in methionine (35). Faba beans though low in the sulfur amino acids,

methionine and cystine have twice the lysine content of hard red spring wheat (23). Of the other amino acids arginine and aspartic acid are high compared to wheat whereas glutamic acid and proline are low (23). The sulfur amino acids and glycine are lower in the cotyledon than in the hull portion of the bean while arginine and glutamic acid are lower in the hull than the cotyledon (49). Kaldy (36) converted the amino acid composition to protein scores to obtain an estimate of protein quality. The scores he reported varied from 36 to 45 for different varieties of faba beans with an average of 40 in comparison to "ideal protein" with a score of 100. Palmer and Thompson (53) reported chemical scores and biological values for four cultivars of Vicia faba to range from 45 - 52 and 45 - 51 respectively. The protein scores verified that methionine is the first limiting amino acid in faba beans. Methionine and cystine values reported for Vicia faba L. range from 0.54 g to 0.95 g/16 g of N for methionine and 0.78 to 1.37 g/16 g of N for cystine (23, 24, 36, 49).

Faba Beans As A livestock Feed

It is generally agreed that the nutritional quality of faba beans is improved if they are first heat treated and if diets containing faba beans are supplemented with methionine. Methionine additions to chick diets containing 87% faba bean improved chick growth rate and feed:gain ratio 110% and 38% respectively (45). Autoclaving faba beans

before being added to chick diets (85% of diet) improved chick growth by 16% and feed:gain ratio by 10% (46). Brisson (9) reported that faba bean diets must be supplemented with animal protein or methionine to have normal growth in chickens. Feeding raw faba beans (Vicia faba L.) (35%) to poultry results in poor growth and feed utilization compared to a control grain diet. Blair, Wilson and Bolton (7) found feeding broilers diets containing increasing levels of faba beans (Vicia faba L.) (0, 15, 30 and 45%) from 0-4 and 0-8 weeks gave significant worsening in food conversion ratio and in weight gain. Wilson and McNab reported that autoclaving only improved chick growth if the sulfur amino acids were limiting and the improvement was possibly due to increased availability of some amino acids (76). Kadérvel and Clandinin (33) found that feeding up to 20% faba beans to chicks had no deleterious effect on growth rate. At 35% there was nonsignificant decrease in weight gain and increased pancreas weight. Autoclaving increased weight gain and decreased pancreas weight. Sharby and Bell (61) comparing soybean meal, rapeseed meal and faba beans found faba beans gave poor growth response. Upon supplementation with methionine the growth response increased to that of soybean meal. Autoclaving alone gave no improved response. Davidson (17) recommends no higher than 15% faba beans be fed in laying hen rations. At 25 to 33% with or without methionine egg production was lowered compared to 15% added faba beans.

Palmer and Thompson (53) state that none of the varieties of faba beans they tested showed any evidence of toxicity when fed to rats (10% crude protein diets with faba beans as protein source). The varieties tested included two field beans (Vicia faba L. var minor), one horsebean (Vicia faba L. var equina) and one broad bean (Vicia faba L. var major). These faba beans were subjected to immediate low temperature after harvesting and were freeze-dried to prevent destruction of any toxins in the bean and to minimize the growth of micro-organisms. They, therefore, believe that poor storage conditions giving rise to fungal and bacterial growth may be a contributary cause of the poor nutritive value attributed to faba beans.

Growth Inhibitors Present in Faba Beans

There are a number of factors that can affect plant protein utilization. These include; enzyme resistant peptides, tightly folded protein conformation, the presence of inhibitors, or a deficiency of one or more essential amino acids (35).

Trypsin Inhibitors

The presence of trypsin inhibitors in faba beans has been reported. Wilson and McNab (78) presented in vitro evidence of the presence of a heat-labile inhibitor of trypsin in both cotyledon and testa of the field bean. Marquardt et al (49) reported a trypsin inhibitor content per unit of dry matter twofold greater in the hull than the

cotyledon of the faba bean (Vicia faba L. var minor). Wilson et al (77) concluded that the trypsin inhibitor does not seem to be responsible for the poor utilization observed upon feeding faba beans to chicks. An extract rich in trypsin inhibiting activity obtained from Vicia faba L. and added to a diet containing autoclaved field bean meal produced no significant effect on chick growth. Pancreas size and food conversion efficiency, however, were reduced. No beneficial effect on the growth of chicks was observed when tannins were removed by ethanol extraction.

Kakade (34) attributed 40% of the growth inhibiting properties of soybeans to trypsin inhibitors. It is known, however, that soybeans contain approximately 5 to 9 fold higher concentration of trypsin inhibitor that faba beans (49, 70). Trypsin inhibitor is therefore of greater metabolic significance in soybeans as compared to faba beans.

Phytohemagglutinins

Phytohemagglutinins (lectins), substances which have the ability to agglutinate red blood cells, have rather high activity in faba beans (40, 49). Lectins isolated from a number of legumes have been shown to be toxic when injected into animals and growth inhibitory when incorported into the diet. All hemagglutinin activity seems to be concentrated in the cotyledon portion of the bean with no hemagglutinin activity in the hull portion. Hemmagglutinin activity ranged from a low of 3.4×10^3 for Herra and Klein-Thuringer to a

high of 5.6×10^3 units per gram whole bean for Erfordia with considerable within cultivar variation (49).

Pusztai (55) indicates that the growth depression associated with the protein from the Phaseolus bean Processor is related to the hemagglutinin content. The poor nutritional performance which other workers have observed on feeding raw faba beans has not been linked conclusively to hemagglutinins (41, 49, 78).

Tannins

The intake of phenols other than tryosine is generally very low in carnivorous animals, whereas herbivores and omnivores may consume considerable amounts of phenol. According to Singleton and Kratzer (62) even man may have high consumption since red table wine contains about 1500 mg per litre of total phenols and accounts for a annual per captia consumption of about 150 g of "tannin" in some societies. Tannins are present in some plant materials at very high levels of 10% or more of the dry wt. (62). They may be of significance in some common feedstuffs such as sorghum grains (14) and rapeseed meal (13). In much of the literature there is considerable generalization in the classification of the substances regarded as tannins. There are two distinctive groups, the hydrolyzable and the condensed tannins. They have in common protein-binding and leather-forming activities, but they usually differ considerably in botanical distribution, other properties, and breakdown products. Tannic

acid is typical of the hydrolyzable tannins. It is readily hydrolyzed enzymatically or hydrolyzes spontaneously to glucose and gallic acid. The condensed tannins (flavolans) are polymeric flavanoids composed predominantly of leucoanthocyanidin units linked carbon-to-carbon from the 4 position of one unit to the 6 - or 8 - position of the next. They do not break down readily under physiological conditions; when treated drastically, they usually produce either less soluble polymeric "phlobaphenes" or flavonoid monomers, particularily catechins and anthocyanidins (63). These phenolic compounds combine with proteins reversibly by hydrogen bonding, and irreversibly by oxidation followed by covalent condensation (42).

Chang and Fuller (14) reported that feeding sorghum grains with high tannin content resulted in growth retardation of chicks similar to that caused by feeding equivalent levels of tannic acid. Fuller et al (25) found that milo sorghum containing 1.6% or more of tannin depressed chick growth when fed as 50% of the diet. Vohra et al (71) reported that as little as 0.5% tannic acid caused a depression in the growth of chicks by reducing the metabolizable energy, feed intake and nitrogen retention. Additional methionine, choline, betaine or ornithine did not prevent the growth depressing properties of tannic acid. Hughes (32) found that a dietary level of 0.05% tannins from sawdust resulted in motted yellow yolks in chicken eggs while 0.5% tannins gave olive green yolks.

Feeding high tannin bird resistant sorghum gave poorer weight gain and feed conversion than bird susceptible low tannin sorghum (58, 59). Extracting tannins from bird resistant sorghum resulted in significantly better chick growth and feed conversion compared to chicks fed intact bird resistant sorghum diets. Extraction treatment of the non-resistant varieties did not significantly influence growth rate but significantly improved feed conversion (2). Additions of methionine and/or PVP (polyvinylpyrolidone) improved chick growth and feed efficiency. Addition of D, L, - methionine (0.15%) to both non-resistant sorghum and resistant sorghum resulted in significant improvement in chick performance. The magnitude of the response was greater with the bird resistant than with the non-resistant sorghum grain diets, however, feed efficiency of the bird-resistant sorghum remained poorer. If tannic acid is added, methionine improves feed:gain ratio from both non-resistant and resistant varieties but has little effect on weight gain with the non-resistant plus tannic acid (1, 3). This data, therefore, seems to indicate that there is no correlation between tannic acid, weight gain and methionine levels in sorghum grain. The effects of adding tannic acid in a protein-free chick diet (1.41%) resulted in a 4 fold increase in endogenous amino acid excretion. The addition of tannic acid to a low tannin sorghum (1.41%) produced a relatively small decrease in apparent amino acid digestibility which could be accounted

for by the increase in endogenous amino acid excretion. Apparent digestibilities of all the amino acids of low, intermediate and high tannin varieties was 73, 41 and 22%, whereas the corresponding value for the low tannin sorghum (59) plus tannic acid was 63%. Stephenson et al (65) reported that amino acid availability in sorghum hybrids varied markedly. Nelson et al (52) found the correlation coefficient for tannin content in sorghum grains to amino acid availability to be highly significant ($P < 0.01$). Also the correlation coefficient for tannin content to kilocalories metabolizable energy per gram (-0.62) and percentage of gross energy utilized (-0.62) were significant ($P < 0.05$). Neither endosperm color nor starch texture appeared to influence amino acid availability or energy utilization.

Tamir and Alumot (70) reported that tannins could be extracted from green and ripe carobs by extracting with hot water and the low molecular weight polyphenols with ethyl acetate. The extracted tannins inhibited α-amylase, trypsin and lipase. Polyvinylpyrrolidone addition reactivated trypsin and lipase but not α-amylase. The inhibitor was found to change the maximum reaction velocity of crystalline trypsin and α-amylase indicating non-competitive reaction kinetics. Feeding green and ripe carobs to rats decreased feed consumption, depressed growth, increased nitrogen excretion and increased the activity of digestive (particularly proteolytic) enzymes in the cecum (70). The decreased

intake was found mostly with green carobs which contain higher amounts of low weight polyphenolic compounds (soluble in ethyl acetate) which possibly caused the astringent taste of the carobs. The tannins in green and ripe carobs not soluble in ethyl acetate did not affect intake much but decreased rat growth. It is thought they form insoluble protein tannin complexes, and therefore reduce the available protein. Feeding increasing amounts of tannins to rats with soybean meal as the protein source demonstrated that tannin exerts a severe negative effect on protein digestibility (22). A determination of amino acid availability did not indicate available methionine to be more severely affected than the availability of the total nitrogen. Available proline, glycine and glutamic acid are severely reduced indicating they may have a specific detoxifying effect. The concentrations of these amino acids are high in gelatin and it is known that tannins and gelatin bind strongly. There is a significant negative correlation between the tannin content in barley and protein digestibility (22). Excess protein in a rat diet increases feed intake and weight gain compared to low protein diet if tannic acid is included. It is thought that one of the deleterious effects of tannic acid is on nitrogen retention, therefore excess protein would partially overcome the loss of nitrogen and the only effect would be then due to palatability. The markedly improved feed intake with protein supplementation may also involve

increased palatability due to decreased astringency of the tannic acid diet. Astringency is thought to result from binding of mucosal proteins in the mouth by tannin and addition of protein counteracts this effect.

Using ^{14}C-labelled casein and by proteolytic enzyme assays of intestinal contents and pancreases it was shown with the rat that protein from enzymatic or other endogenous origins constitute a large portion of the excreted nitrogen compounds (27).

Melić et al (50) isolated tannins from lucerne and found in "in vitro" studies the isolated hydrolyzable tannin had a high inhibitory action on digestive enzymes. Strumeyer and Malin (66) isolated tannins from Leoti red sorghum and Georgia 615 on LH-20 Sephadex. The tannins from both strains were shown to consist of a series of polymeric polyphenols which upon acid hydrolysis generated cyanidin exclusively. Bond (8) has shown that white-flowered tannin free varieties of Vicia faba show increased "in vitro" digestibility compared to coloured-flowered varieties of similar seed size. The difference was shown to be largely due to the mean D value (digestibility of the organic matter) of the testa in tannin-free varieties of 56.4% compared with 17.2% in tannin-containing varieties.

MATERIALS AND METHODS

Preparation of Faba Bean Fractions

Diana faba beans were used in most of the experiments except when various varieties were compared. The beans were fed as whole ground beans or as a component of the whole bean. The bean was separated into its components, by cracking the hull with a platetype grinder, followed by mechanical removal of the hulls. The dehulled faba beans (cotyledon) were separated into protein and starch fractions in a commercial plant, utilizing a process of air classification (16).

General Bird Management and Diet Formulation

Day-old male broiler or leghorn chicks were obtained from commercial hatcheries. They were housed for all experiments, in electrically-heated, thermostatically-controlled batteries with raised wire floors. Prior to the initiation of the experiments the birds were starved for 4 hours, weighed individually and allocated to pens. The experimental diets were fed ad libitum and the birds had continual access to water with constant lighting. The birds were weighed at the termination of each experiment after a 4 hour fast. At the termination of particular experiments, liver, pancreas and/or spleen were immediately excised, dissected free of

fat and other material and weighed. Usually 2 to 3 birds per pen were examined.

Analysis of Faba Bean Fractions and Diets Gross Composition

Dry matter, crude protein (N x 6.25), fat (ether extract), crude fibre, acid detergent fibre and ash were determined by methods according to the Association of Official Agricultural Chemists (4). The determination of chromic oxide was by the method Williams (75).

Trypsin and Hemagglutinin Assay

Extracts for trypsin inhibitor and hemagglutinin assays (49) were prepared by adding 1 g of finely ground sample to 20 ml of 0.85% Na cl. The suspension was stirred for 30 minutes at 30°, allowed to stand an additional 90 minutes at 30°, and then overnight (18 hours at 2°). The suspension was centrifuged at 50,000 x g for 15 minutes and the supernatant was either assayed immediately or stored at -20° until required. Preliminary studies demonstrated that both trypsin inhibitor and hemagglutinin activities were not altered when stored at -20° after a period of up to 1 month. The trypsin inhibitor activity of faba beans and soybeans was determined spectrophotometrically using a modification of Kassell's method (37). The principle of the assay involves a measurement at 405 nm of the inhibition of trypsin hydrolysis of N - α - benzoyl - DL - arginine - p - nitroanilide (BAPA). Modifications of the above assay included

the following: incubation temperature (30°), method of preparing trypsin inhibitor, buffer concentration, substrate concentration and order of addition of reagents to the reaction mixture. Trypsin inhibitor was prepared by dissolving 10 mg of trypsin in 50 ml of 0.001 N HCl - 0.01 M Ca Cl_2. Trypsin inhibitor stability is pH-dependent. Maximum stability is achieved in the pH range of 3-4. The substrate was prepared by dissolving, with constant mixing, 100 mg BAPA in 80-85 distilled H_2O. During the assay period this solution was maintained at 30°, and during storage at 3°. The substrate was stable for 2 weeks at 3°. The assay was carried out in 1 cm cuvettes in a Gilford Model 2400 recording spectrophotometer. One millilitre of 0.2 Molar Tris., 0.02 Molar Ca Cl_2 (pH 8.2 at 30°), 0.2 ml. of trypsin (40 ug) and 0.2 ml of inhibitor solution were added to the cuvette and were incubated for 5 minutes at 30°. The reaction was initiated by the addition of 1.5 ml of substrate (kept at 30°) and was followed for 4-5 minutes. Lima bean protease inhibitor was used as a standard. A unit of inhibitor is defined as the amount of inhibitor that will inhibit the enzymatic activity of 1 mg of active trypsin in the above assay. The hemagglutinin test was similar to that reported by Liener (40) except that the erythrocytes were not treated with trypsin, incubation temperatures were more rigorously controlled, and the cells were resuspended 10 minutes prior to being read at 620 nm. All reagents were

kept in an ice bath prior to initiation of the reaction. The reaction mixture was incubated at 30° for 4 hours and then placed in ice for 30 minutes followed by incubation overnight at 2° . One unit of hemagglutin activity is defined as the level of test solution which cause 50% of the standard cell suspension to sediment. Rabbit red blood cells were used for analysis.

Other Analyses

Vicine was determined by the method of Higazi and Read (31). The degree of gelatinization of processed faba beans was determined as amount of maltose released after incubation with beta-amylase (67). Amino acid analyses were performed by the method of Moore and Stein (51) with a Beckman automatic amino acid analyzer. Ninhydrin analysis was by the method of Rosen (57). Assays for condensed tannins were carried out according to the vanillin-hydrochloric acid method of Burns (10). The standard for this test was purified fraction B from the Sephadex LH-20 column (pure condensed tannin) obtained from the faba bean hull extract. The absorbance of this standard was 3.5 fold greater than a standard catechin sample obtained from Sigma Chemical Company. Lactate dehydrogenase assay was by the method of Bergmeyer et.al.(5). Analysis of variance was conducted according to

Snedecor (64) and treatment differences were subjected to the Students-Newman-Keuls multiple range test as outlined by Kirk (89).

The Effect of Heat Treatment on Nutritional Quality of Faba Beans

Comparison of Various Heating Methods

The heat treatments studied were microwave irradiation, steam pelleting, extrusion, and autoclaving. The composition of the basal diet is shown in Table 1 (Experiment 1A). Microwave irridation (1,600 watts) of ground beans was carried out in 7 x 40 x 40 cm cardboard trays in a Raytheon Radarange oven for either 20 or 30 minutes. The average interior temperature of the layers of faba beans in the trays were 101° and 107° after the 20 and 30 minute treatment periods respectively. Steam pelleting was carried out at 70° in a commercial pelleter (California Master Model Pellet Mill). Faba beans were extruded at 130° and 152° (Brandy Crop Cooker, Koehring). Prior to extrusion, 7.5% oil was added to the ground beans. Autoclaving of the ground beans was carried out at 121° for 20 minutes. The beans were spread in porcelain trays to a depth of 2 cm. The pelleted and extruded faba beans were reground prior to the initiation of the experiment.

Effect of Moisture Content on Microwave Irradiation

Varying amounts of water (0, 8% or 18%) were added to ground faba beans. The faba beans were then subjected to

microwave (1,600 watts) treatment for 0 and 26 minutes in 7 x 40 x 40 cm cardboard trays. Ingredient additions were made on the basis of a 10% moisture content in faba beans. The basal diet is shown in Table 1 (Experiment 1B).

Autoclaving Time on Nutritional Quality of Faba Beans

Ground faba beans were spread to a depth of 2 cm in porcelain trays and autoclaved at 121° for varying periods of time (0, 3, 10, 20, 30 and 40 minutes). The basal diet used in this experiment is shown in Table 1 (Experiment 1C).

Identification of the Major Growth Inhibiting Fraction of the Faba Bean

Effect of Heating the Faba Bean Starch Fraction Compared to Heating the Protein Fraction of the Faba Bean

Faba bean starch and faba bean protein were autoclaved fro 20 minutes as described above. In addition, another sample of faba bean starch was extruded at 132° and then ground. Prior to extrusion, 10% soybean oil was added to the starch fraction. The basal diet is shown in Table 2 (Diet A).

Comparison of the Growth Inhibition of the Protein and Hull Portions of Faba Beans

Experiment 1 Diets were prepared with varying amounts of hull content (Table 2, Diet B). The percent hull was calculated from the crude fibre analysis of pure preparations of faba bean hulls -53% crude fibre (49) and faba bean cotyledon -1.4% crude fibre (49) and the corresponding

TABLE 1. Formula and analyses of basal diets fed to chicks to determine the effect of heat treatment, moisture level and heating time on faba bean and its components.

Ingredients	Experiment 1A	Experiment 1B	Experiment 1C
	%	%	%
Faba beans[1]	87.5	92.6	85
Faba bean starch	--	--	7.6
Faba bean protein isolate	--	--	--
Soybean oil	7.1	2.0	2.0
Calcium phosphate	2.4	2.4	2.4
Calcium carbonate	1.0	1.0	1.0
Vitamin premix[2]	1.0	1.0	1.0
Mineral premix[3]	0.5	0.5	0.5
Methionine premix[4]	0.5	0.5	0.5
Chemical Analyses of diets[5]			
Dry matter	91.7±0.9	90.0	90.5±0.6
Protein (N x 6.25)	24.0±0.2	26.4±0.1	24.2±0.4
Fat (ether extract)	7.8±0.1	2.9±0.1	3.4±0.2
Crude fiber	6.7±0.2	7.2±0.2	8.1±0.1
Ash	6.1±0.1	5.3±0.2	5.8±0.1

[1]Cultivars of faba beans used for Experiment 1A and 1C were Diana and for Experiment 1B, Ackerperle. Proximate analyses for the Diana and Ackerperle faba beans were dry matter, 91% and 90%; protein (N x 6.25) 28.2% and 28%; fat (ether extract) 0.9% and 0.9%; and crude fiber 7.9% and 7.8% respectively.

[2]The vitamin mixture per kg of diet was: retinyl palmitate, 7,500 IU; cholecalciferol, 1,000 ICU; α-tocopherol, 10 IU; menadione, 2.2 mg; thiamine, 2.2 mg; riboflavin, 4.4 mg pantothenic acid; 14.3 mg; niacin, 33 mg; pyridoxine, 4.4 mg; biotin, 0.13 mg; folic acid, 1.3 mg; choline chloride, 1,320 mg; vitamin B_{12} 0.011 mg; and anti-oxidant (Santoquin), 250 mg.

Cont'd.....

[3]The composition of the mineral mix (mg/kg of diet) was: manganese, 16 as MgO; zinc, 1.4 as ZnO; iron, 3.1 as $FeSO_4 \cdot 7H_2O$ copper, 2.5 as $CuSO_4 \cdot 5H_2O$; and iodized NaCl, 4,930.

[4]Methionine added per kg diet was 3.0 grams.

[5]In experiment 1B analysis was calculated on the basis of 90% dry matter. The matter content of the diets containing faba beans with 0%, 8% and 18% H_2O added were 92%, 85% and 78% respectively. All diets from experiment 1B were stored at 20° to prevent spoilage.

TABLE 2. Formula and analysis of diets fed to chicks to determine in what fraction of the faba bean the major growth inhibitor is located.

Ingredients[1]	Diet A[2]	Diet B[3]			Diet C[4]
		Dehulled	Whole	Whole & Hulls	
	%	%	%	%	%
Whole faba beans	--	--	83.1	74.8	--
Dehulled faba beans	--	75.0	--	--	--
Faba bean protein	34.9	--	--	--	30
Faba bean starch	51.0	17.6	9.5	--	--
Faba bean hulls	--	--	--	17.8	30
Soybean oil	2.4	2.0	2.0	2.0	8.0
Other ingredients	5.4	5.4	5.4	5.4	32
Chemical analyses of diets diets[5]					
Dry matter	88.4±0.2	91.4±0.1	91.4±0.1	91.4±0.1	91.9±0.4
Protein (Nx6.25)	22.3±0.3	23.3±0.1	22.2±0.1	21.8±0.1	22.9±0.4
Fat (ether extract)	9.2±0.4	3.1±0.1	3.4±0.1	3.3±0.1	9.3±0.4
Crude fiber	1.6±0.1	1.9±0.1	7.5±0.1	11.9±0.1	14.0±0.4
Acid detergent fiber	--	3.0±0.1	10.4±0.2	16.9±0.2	--
Ash	5.2±0.2	5.6±0.1	6.0±0.1	6.0±0.1	6.05±0.4

[1]See Materials and Methods for preparations of fractions. The beans used were Diana. Other ingredients for experiments A, B and C were calcium phosphate 2.4, 2.4 and 0%; mineral mix 0.5, 0.5 and 0.5%; analyses for raw and heated diets ± SEM. [2]After formulation of the diets, 5% H_2O was added to improve texture. [3]The diets were of the same composition either raw or autoclaved. [4]Four combinations of faba bean protein concentrate and hull were used; see Results, Table 12.

crude fibre analysis of the faba bean containing diets. Dehulled ground faba beans (1% hulls), whole ground faba beans (13% hulls), ground faba bean hulls (58% hull plus 42% cotyledon) and faba bean starch (1% hulls) were each divided into two equal parts. One part was heat treated and the other was not. Heat treatment consisted of spreading the beans to a depth of 2 cm in porcelain trays and autoclaving at 121° for 30 minutes.

Experiment 2 Faba bean protein concentrate and hulls were prepared as described above. The hulls were the same as those used in previous experiments except they were further cleaned by means of a mechanical seed cleaner. The purity of these hulls as estimated from the relative protein concentration of a pure preparation of hulled and dehulled beans, was 96%. The protein concentrate and hull fractions were subdivided into two equal parts; one-half was not treated and one-half was autoclaved at 121° as described above. They were then mixed with a basal corn diet (Table 2C and 12), and 7% water was added to increase palatability. In trial 1 the diet was fed as a mash whereas in trial 2 the diet was pelleted.

Isolation of the Growth Inhibiting Factor From the Faba Bean Hull

Experiment 1 Faba bean hulls (96% pure hulls) were divided into two portions. One portion was not treated. The other portion of the hulls was extracted with 7 volumes of

distilled water per kilogram of hull for 6 hours at 23° with stirring every 30 minutes. After 6 hours, the supernatant was passed through several layers of cheesecloth. The extracted hulls were rewashed several times until the filtrate was clear. These extracted hulls were spread to a thickness of 1 cm and allowed to dry at 30° for 2 days. The extracted and non-extracted hulls were subsequently divided into two groups each; one group from each was autoclaved at 121° for 30 minutes. The other two groups were not treated. Four combinations of raw and autoclaved faba bean hulls, either non-extracted or extracted were incorporated into four diets (Table 4).

Experiment 2. Faba bean hulls were extracted following the procedure described above, except the hulls were re-extracted immediately with a further 7 volumes of distilled water and the two extracts combined. The combined extract was concentrated using a Ajax International Corporation reverse osmosis apparatus followed by evaporation with a cyclone evaporator and then lyophilized. The amount of sample extracted from the hulls was determined to be 6.4% ± 0.3. Since the hull makes up 13% of the seed, the concentration of the extracted material was 0.83% of the whole seed. The extract was divided into two equal parts, one part was un-treated and the other part was autoclaved at 121° for 30 minutes. Two levels of the extract either raw or autoclaved were incorporated into corn-soybean meal diets and were fed

along with two control diets (Table 5).

Identification of the Growth Inhibitor

To Determine the Effect of PVP (Polyvinylpyrolidone) on the Growth Inhibitor in the Faba Bean Hull Extract

Chick Growth Trial The water extract was prepared by extracting the hulls twice with 7 volumes of distilled water as described above. Soluble PVP (Sigma Chemical Company) used in the experiment had a average molecular weight of 10,000. The basal diet used in the experiment is shown in Table 3.

PVP Column Chromatography A 90 x 9.5 cm column was packed with insoluble PVP. The hull water extract was prepared as above. The column was eluted with distilled water and then with 0.2 N NaOH.

Methods Used to Concentrate the Growth Inhibitor

Acetone-Water Fractionation of the Faba Bean Hull Water Extract

Faba bean hulls were extracted twice with 7 volumes of distilled water as described above. The crude extract was subjected to a freeze-thaw cycle which denatured much of the extracted protein. The resulting clear solution was separated from the precipitated material by decantation and was concentrated 10 fold over a period of 1 day by reverse osmosis. The extract was further concentrated 2 fold under reduced

TABLE 3. Formula of basal diets.

Ingredients	% Added
Corn	45.0
Soybean oil meal	37.7
Tallow	4.0
Calcium phosphate	2.5
Calcium carbonate	0.8
Vitamin premix[1]	1.0
Mineral premix[1]	0.5
Methionine and chromix oxide premix[2]	0.5
Other ingredients[3]	8.0

[1]The vitamin and mineral premixes were as described in Table 1.

[2]Methionine and chromic oxide added per kg diet were 8g and 2g respectively.

[3]See Materials and Methods, and Results for a description of other ingredients added. The proximate analysis of the basal diet minus the 8% of other ingredients was: dry matter, 91.3±0.1%; protein (N x 6.25), 23.4±0.1%; fat (ether extract), 6.8±0.1%; crude fiber, 2.5±0.1%; and ash, 6.4±0.1%.

TABLE 4. Formula and analyses of faba bean hull diets.

Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
	%	%	%	%
Faba bean hulls, raw[1]	40			
Faba bean hulls, autoclaved		40		
Faba bean hulls, extracted			40	
Faba bean hulls, extracted and autoclaved				40
Other ingredients[2]	60	60	60	60
Chemical analyses of diets[3]				
Dry matter	92.9±0.1	93.0±0.1	92.5±0.1	92.2±0.1
Protein (N x 6.25)	19.7±0.1	19.7±0.1	18.7±0.1	19.1±0.1
Fat (ether extract)	7.6±0.2	7.4±0.1	7.4±0.1	7.5±0.1
Crude fiber	18.8±0.1	19.1±0.1	20.1±0.2	19.2±0.1
Ash	6.3±0.1	6.3±0.1	6.0±0.1	5.8±0.1

[1]See Materials and Methods for preparation of hull fractions.

[2]The percent of the other ingredients were: corn 18.5%; soybean oil meal 30.2%; rapeseed oil 6.0%; calcium phosphate 2.5%; calcium carbonate 0.8%; vitamin premix 1.0%; mineral premix 0.5%; and amino acid-chromic oxide premix 0.5%. The composition of the vitamin and mineral premix was as described in Table 1. Methionine and chromic oxide were at a level of 1.3 and 2.0 g per kg diet, respectively.

[3]The average proximate analyses ± SEM of raw and extracted hulls were: dry matter 93.2±0.2 and 95.4±0.1; protein (N x 6.25), 7.8±0.1 and 6.6±0.1; fat (ether extract), 0.4±0.1 and 0.4±0.0; crude fiber, 44.6±0.5 and 51.7±0.2; and ash 2.79±0.02 and 1.30±0.2, respectively. Analysis represents average ± SEM of duplicate samples.

TABLE 5. Formula and proximate analyses of diets containing water extract of faba bean hulls.

Ingredients	Diet Number					
	1	2	3	4	5	6
Sucrose	4.8				2.4	2.4
Cellulose[1]		4.8				
Water extract of hulls, raw[2]			4.8		2.4	
Water extract of hulls, autoclaved[2]				4.8		2.4
Other ingredients[3]	95.2	95.2	95.2	95.2	95.2	95.2
Chemical analyses of diets[4]						
Dry matter	92.4±0.1	92.3±0.01	92.6±0.01	92.2±0.01	91.9±0.01	92.3±0.01
Protein (N x 6.25)	23.8±0.1	23.8±0.1	24.2±0.1	24.0±0.1	23.9±0.1	23.9±0.1
Fat (ether extract)	6.9±0.1	7.0±0.1	6.8±0.1	6.9±0.1	6.9±0.1	7.1±0.3
Crude fiber	2.8±0.1	7.3±0.1	2.5±0.1	2.5±0.1	2.6±0.1	2.6±0.1

[1]Alfa-floc, commercial cellulose from Brown and Co.

[2]Procedures for the preparation of the lyophilized water extracts of hulls and for autoclaving are described in Materials and Methods.

[3]The percent of other ingredients were: corn, 47.5%; soybean oil meal, 38.7%; tallow 4.0%; calcium phosphate, 2.5%; calcium carbonate, 0.8%; vitamin premix, 1.0%; mineral premix, 0.5%; and amino acid premix, 0.2%. The composition of vitamin and mineral premixes was as described in Table 1. The amino acid premix was equivalent to 0.8 g added methionine per kg of diet.

[4]The average proximate analyses ± SEM of the lyophilized water extract of hulls were: dry matter, 92.3±0.7%; protein, 8.5±0.7%; fat (ether extract), 0.2±0.2%; crude fiber, 0.1% (single analysis); ash 12.1±0.2%. All other analyses represent means ± SEM for duplicate or triplicate samples.

pressure and finally to dryness by lyophilization. The dried product was stored at - 20° until required. The dry faba bean hull water extract prepared was dissolved in distilled water in the ratio of 1 g of extract to 4 mls water. The mixture was centrifuged at 50,000 x g for 20 minutes and then re-extracted with the same volume of water and recentrifuged and the extracts combined. This yielded two fractions; one insoluble in water and the other soluble. Acetone was added to the soluble fraction, so the final ratio of acetone to water was 75:25. This mixture was stirred and allowed to stand 10 minutes then centrifuged as above. The precipitate was washed with 75:25 acetone-water and recentrifuged. The supernatant solutions were combined. This yielded two fractions; one soluble in 75:25 acetone-water and the other insoluble. Acetone was added to the soluble fraction so the final acetone to water ratio was 90:10. The mixture was centrifuged and re-extracted with 90:10 acetone-water as above and the supernatants combined. This yielded two fractions; one soluble in 90:10 acetone-water and the other insoluble. The precipitates were dried under reduced pressure to remove the solvent and then lyophilized. The supernatants were concentrated by means of a cyclone evaporator and then lyophilized. Of the original water extract the water insoluble fraction, the 75:25 acetone-water insoluble fraction, the 90:10 acetone-water insoluble fraction and the 90:10 acetone-water soluble fraction

represented 10, 27, 16 and 45% of the dry material respectively. The total recovery was 98%. All fractions were stores at -70° until required.

Sephadex LH-20 Chromatography of Faba Bean Hull Extracts

A lyophilized water extract of faba bean hulls was prepared as described. The dried extract was dissolved in four volumes of distilled water and allowed to mix 30 minutes. Nine volumes of acetone per volume of water was added so the final ratio of acetone to water was 90:10. This procedure, which is a simplication of the acetone fractionation procedure, yielded two fractions, a soluble and insoluble fraction. The insoluble material adhered to the walls of the container and settled to the bottom upon standing 16 hours at 2°. The supernatant was poured off and yielded a product equal to 40% of the original weight. The material was dried as above and stored at -70° until required.

A portion (38 g) of the 90:10 acetone-water lyophilized powder was dissolved in 150 ml of 90% ethanol and applied to a Sephadex LH-20 column (32 x 6.5 cm) previously equilibrated with 95% ethanol. The column was developed with 95% ethanol (5,000 ml) and then 50:50 acetone-water (1,000 ml). The column was eluted at a flow rate of 60 ml/hour and the eluent was monitored at 280 nm (ethanol effluent) and 400 nm (water-acetone effluent) with a Gilford recording spectrophotometer (model 2400). The effluents were pooled into three fractions: fraction A which gave a

negative $FeCl_3$ test for condensed tannins (i.e. no precipitate formed with 10% $FeCl_3$ solution), an intermediate fraction eluted with 95% ethanol which gave a slight precipitate with $FeCl_3$, and fraction B (eluted with acetone-water) which gave a very pronounced precipitate with $FeCl_3$. The samples were concentrated and stored as above. The intermediate fraction was combined with fraction A.

Properties of Faba Bean Condensed Tannins and Low Molecular Weight Polyphenolics

Analytical Resolution of Tannin and Non-Tannin Components

A portion of fraction A and B and the 90:10 acetone-water extract were either fractionated or refractionated on an analytical scale so as to assess the degree that the above described preparative procedure was able to resolve the acetone-water extract into tannin and nontannin components. This procedure was as described above except the LH-20 column size was reduced (20 x 2 cm) and the load facter (g of sample applied per unit volume of column) was approximately 7 fold less. The amount of sample applied to each column was 0.4 g in 4 ml of 90% ethanol.

Chemical Characterization of Fraction A and B

An absorption spectrum in 0.2 N NaOH and 0.2 N NaCl was determined using a double beam recording spectrophotometer (Unicam SP 800). The concentration of the aqueous $FeCl_3$ solution was 10% w/v.

Thin Layer Chromatography of Faba Bean Hull Fractions

Cellulose plates 200 x 200 mm, 0.25 mm thick were applied with the aid of a quickfit TLC assembly (Model 8CR) using a slurry of cellulose powder, (cellulose, microcrystalline, Baker TLC reagent) and water in the proportions 15:80. Two solvents were employed: BAW (butan - 1 - ol, acetic acid and water in the proportions 4:1:2.2) and BEAW (butan - 1 - ol, ethylmethylketone, acetic acid and water in the proportions 2:5:1:2). The samples were applied to the plates in 90% acetone solution and volumes of from 10 to 40 ul were used. Forced air drying of the sample spots was omitted to reduce danger of oxidation. Each sample was applied at the corner of a plate approximately 15 mm from the bottom and 30 mm from the adjacent edge. Each plate was developed in BAW solvent until the solvent front was about 10 mm from the top of the plate. The plate was then removed from the tank, allowed to dry under a fume hood and then developed in a direction perpendicular to the first in BEAW solvent. After the plates had been dried from the second development, they were sprayed with ferric chloride/potassium ferrocyanide reagent Kirby et al. (38). This spray shows phenolic compounds as blue coloured areas against a pale yellow background.

RESULTS

I. The Effect of Heat Treatment on the Nutritional Quality of Faba Beans

Comparison of Various Heating Methods

Marquardt and Campbell (44, 46) have demonstrated there is a significant increase in weight gain and decrease in feed intake and in feed:gain ratio and pancreas size when birds are given a diet containing high levels (85-90%) of autoclaved (121°) faba beans as compared with a diet containing the same level of raw faba beans. Similarily, Wilson and McNab (76) showed that autoclaving a diet containing 75% faba beans resulted in a beneficial effect on liveweight gain and feed conversion efficiency.

In the present study, different methods of heat treatment of faba beans were compared as to their effectiveness in promoting improved chick growth, (Table 6). Microwave treatment was the least effective and autoclaving or extruding were the most effective of the various processing methods. The corresponding increases in weight gain and the improvements in feed:gain ratios for chicks fed processed faba beans as compared with raw faba beans were: microwave treatment for 20 minutes, -1% and -4%;

TABLE 6. Effect of various heat treatments on the utilization of faba beans by broiler chicks.[1]

Treatment of faba beans.	Weight Gain	Feed:Gain Ratio	Liver Weight	Pancreas Weight	Spleen Weight
	kg	g/g	g/kg	g/kg	g/kg
Control[3]	541[c2]	2.01[a]	27	3.2[a]	1.4
Microwave (20 min)	535[c]	2.09[a]	26	2.7[bc]	1.4
Microwave (30 min)	571[bc]	1.99[a]	26	2.6[c]	1.4
Steam Pelleted (70°)	572[bc]	1.85[b]	25	3.0[ab]	1.5
Extruded (130°)	602[ab]	1.66[c]	24	2.8[bc]	1.4
Extruded (152°)	626[a]	1.62[c]	25	2.6[c]	1.5
Autoclaved (121° - 20 min)	632[a]	1.80[b]	26	2.4[c]	1.5
SEM.	11	0.03	1	0.1	0.1

[1]Number of pens in each treatment group was nine. Number of birds per pen was five. Average initial bird weight was 50.6±0.3 SEM. The experiment was initiated when the birds were 3 days of age and was terminated 25 days later.

[2]Means for each response criteria not sharing a common superscript letter within a column were significantly different at $P < 0.01$.

[3]See Materials and Methods (Table 1A) for the basal diet used in the experiment.

TABLE 7. Effect of processing of faba beans on the level of trypsin inhibitor, hemagglutinin and vicine, and degree of gelatinization of starch.

Treatment of faba beans	Trypsin Inhibitor	Hemagglutinin Activity	Vicine	Gelatinization
	mg/g	units[2]	OD660nm[3]	mg maltose/g
Control	1.8±0.3[1]	2700±400	0.11±0.01	106±3
Microwave (20 min)	0.3±0.1	1300±400	0.11±0.01	95±6
Microwave (30 min)	0.2±0.1	1300±400	0.12±0.01	100±4
Steam Pelleted (70°)	1.5±0.1	2700±400	0.11±0.01	108±1
Extruded (130°)	0.4±0.1	300±150	0.12±0.01	180±1
Extruded (152°)	0.2±0.1	200±28	0.13±0.01	250±2
Autoclaved (121°)	0.2±0.1	40±24	0.11±0.01	108±6

[1]Mean ± SEM based on duplicate samples.

[2]One unit of hemagglutinin activity is the level of test solution which causes 50% of the standard rat or rabbit red blood cell suspension to sediment.

[3]Arbitrary units from spectrophotometer demonstrating relative differences between treatments.

microwave treatment for 30 minutes, 6% and 1%; steam pelleting, 6% and 8%; extruding at 152°, 16% and 18% and autoclaving, 17% and 10% respectively. The various processing methods which represented varying degrees of heat treatment had no influence on chick liver or spleen weight or on the vicine content of faba bean but did influence chick pancreas size as well as the activities of trypsin inhibitor and hemagglutinin (Table 6 and 7). There was a positive association between the decrease in chick pancreas size and the activity of trypsin inhibitor in the processed faba beans.

Effect of Moisture Content on Microwave Irradiation

Data presented in Table 8 indicate that the moisture content of faba beans was critical with regard to the response of chicks fed microwave irradiated as compared with raw faba beans. When expressed as a percent of the values for the raw faba bean treatment groups, values for the heat treated groups at 0, 8 and 18% water, respectively, where 100%, 104% and 108% for bodyweight gain and 102%, 83% and 76% for pancreas size. With regard to body weight gain, water content of the faba beans had a positive effect although no response was noted for pancreas size. This increase in body weight can be probably attributed to an increased feed consumption by the birds in response to an improved palability of the diet. Food consumption data,

TABLE 8. Effect of microwave treatment and faba bean moisture content on the utilization of faba beans by broiler chicks.[1]

Microwave time and water added	Chick Growth Response		
	Weight Gain	Liver Weight	Pancreas Weight
	g	g/kg	g/kg
0% water, 0 minutes	478±10	34±1	3.34±0.1
0% water, 26 minutes	477±10	33±1	3.39±0.1
8% water, 0 minutes	482±15	34±1	3.48±0.1
8% water, 26 minutes	499±8	36±1	2.89±0.2
18% water, 0 minutes	491±9	34±1	3.63±0.1
18% water, 26 minutes	531±3	34±1	2.75±0.1

SUMMARY OF ANALYSIS OF VARIANCE

Source	df	Weight Gain Mean Square	F	Liver Weight Mean Square	F	Pancreas Weight Mean Square	F
% water	2	85840	7.12**	4.31	0.62	0.13	1.39
microwave time	1	78307	6.50**	1.62	0.23	2.00	2.22**
% water x time	2	32784	2.72*	9.88	1.42	0.69	7.67**
Error	30	12052		6.79			

*$P < .05$

**$P < .01$

[1]Initial average weight per bird was 112±0.8 SEM. The experimental design was a 3 (moisture level) x 2 (time of microwave treatment) factorial with six replicates of five birds each. The experiment was initiated when the birds were 9 days of age and was terminated 20 days later.

however, have not been presented due to the fact that the moisture content of the orts was not recorded and hence accurate data were not available.

Effect of Autoclaving Time on the Nutritional Quality of Faba Beans

Date presented in Table 9 illustrate the relation between the time interval of heat treatment (autoclaving at 121°) of faba beans and the growth response of broiler chicks, chick liver and pancreas sizes and certain components of faba beans. The maximum improvement in the nutritional value of the faba bean as measured by chick weight gain and feed utilization data occurred after 30 to 40 minutes of heat treatment. In contrast, chick pancreas size and the content in the faba bean of the potential anti-nutritional factors, trypsin inhibitor or hemagglutinin, approached minimal levels after 10 to 20 minutes of heat treatment. Furthermore, chick liver weight, faba bean vicine levels or degree of starch gelatinization were not affected by duration of the heat treatment period.

II. Identification of the Major Growth Inhibiting Fraction of the Faba Bean

Effect of Heating the Faba Bean Starch Fraction Compared to Heating the Protein Fraction of the Faba Bean

The faba beans were dehulled and separated into starch and protein fractions by a commercial process (see Materials and Methods). A experiment was conducted to compare the

TABLE 9. Effect of autoclaving time of faba beans on the level of certain components in faba beans and on the performance of chicks fed autoclaved faba beans.

	Autoclaving Time						
	0 min	3 min	10 min	20 min	30 min	40 min	
Faba bean component							
Gelatinization (mg maltose/g)	130±2	120±3	120±1	120±1	120±2	120±5	
Vicine (OD650nm)[1]	0.11±0.01	0.11±0.01	0.00±0.01	0.11±0.01	0.11±0.01	0.11±.01	
Hemagglutinin Activity (units)[1]	2,690±0	2,100±45	1,350±134	81±0	0±0	0±0.0	
Trypsin Inhibitor (units)[1]	1.00±0.1	0.40±0.01	0.11±0.06	0.10±0.01	0.04±0.01	0.04±0.01	
Bird Performance[2,3]							SEM
Initial weight (g)	91	91	91	91	91	91	0.2
Weight gain (g)	330[b]	321[b]	333[b]	364[ab]	385[a]	383[a]	12
Feed:gain ratio (g/g)	3.18[a]	3.26[a]	3.14[a]	2.84[b]	2.66[b]	2.66[b]	0.1
Liver weight (g/kg)	25	26	25	25	25	25	.4
Pancreas weight (g/kg)	4.0[a]	3.7[a]	3.2[b]	3.2[b]	2.9[b]	2.9[b]	.1

[1]Units for vicine are direct reading from optical density reading from spectrophotometer. One unit of hemagglutinin activity is the level of test solution which causes 50% of the standard rat or rabbit's red blood cell suspension to sediment. One unit of trypsin inhibitor is the amount of inhibitor that will inhibit the enzymatic activity of 1 mg. of active trypsin in a assay. Lima bean protease inhibitor was used as the standard. [2]Number of pens in each treatment group was eight. Number of birds per pen was five. The experiment was initiated when the birds were 6 days of age and was terminated 20 days later. [3]Means for each response criteria not sharing a common superscript letter within a row were significantly different at $P < 0.05$.

influence of heating faba bean starch and protein either alone or in combination on broiler chicken growth response. The effects of heat treatment on chick growth as indicated by body weight gain and efficiency of feed utilization was associated primarily with heat treatment of the protein fraction as compared to the starch fraction (Table 10). The response of chicks fed extruded and autoclaved faba beans was similar but the degree of starch gelatinization was very different (Tables 6 and 7). Although in the above mentioned experiment, birds fed the extruded faba bean diet as compared with the autoclaved faba bean diet utilized feed more efficiently it is probable that this effect would not have been noted if the autoclaving time had been 30 to 40 minutes rather than 20 minutes. The results of these experiments would suggest that the improved utilization of faba bean following autoclaving or extrusion is not associated with an effect on the starch component of the bean. Also gelatinization of the starch component is not a prerequisite for improved utilization of the starch.

Comparison of the Growth Inhibition of the Protein and Hull Portion of the Faba Bean

Experiments were conducted to determine if the major portion of growth inhibitor was concentrated in the protein and/or hull fraction of the bean. In the first experiment three faba bean diets were compared (Materials and Methods Table 2B): ground dehulled faba beans, ground whole faba

TABLE 10. Performance of broiler chicks fed raw and/or autoclaved protein and starch isolates from dehulled faba beans.

Treatment of faba bean fractions[1]	Trial I			Trial II		
	Weight Gain	Feed Intake	Feed: Gain Ratio	Weight Gain	Feed Intake	Feed: Gain Ratio
	g	g		g	g	
Raw protein, raw starch	138^{B2}	284^{ab}	2.07^{A}	242^{Bbc}	356	1.47^{Aa}
Autoclaved protein, raw starch	181^{A}	295^{a}	1.63^{B}	262^{Aa}	355	1.36^{Bc}
Raw protein, autoclaved starch	131^{B}	270^{b}	2.06^{A}	244^{Bbc}	346	1.42^{Ab}
Autoclaved protein autoclaved starch	171^{A}	280^{ab}	1.64^{B}	250^{ABb}	340	1.36^{Bc}
Raw protein, extruded starch	--	--	--	234^{Bc}	341	1.46^{Aa}
SEM	4.6	5.6	0.04	4	5	0.02

[1]Number of pens in each treatment group for each trial was 10. Number of birds in each pen was five. Average initial bird weight was 94 ± 0.8g SEM for Trial I and 52 ± 0.4g SEM for Trial II. The trials were initiated when the birds were 8 (trial 1) and 3 (trial 2) days of age and were terminated after 9 (trial 1) and 13 (trial 2) days.

[2]Means for each response criteria not sharing a common superscript letter with column were significantly different. Uppercase letters denote $P < 0.01$; lower case letters denote $P < 0.05$.

beans plus added hulls either raw or autoclaved. The corresponding level of hulls in the diets were 1, 11 and 20% respectively (see Materials and Methods for calculation of hull levels). Weight gain and feed:gain ratio were markedly improved when birds were fed autoclaved as compared with raw faba beans and when the hull content of the diet was decreased ($P < 0.01$) (Table 11). Feed intake was not affected by processing ($P > 0.05$) but was affected by the level of hulls in the diets. Decreasing the hull content in the diet from 20% to 1% improved weight gain and the feed:gain ratio a maximum of 26% and 33% respectively. Heat treatment of dehulled faba beans increased weight gain by 8% and decreased feed:gain ratio by 8%. In contrast, heat treatment of whole faba bean increased weight gain 15% and feed:gain ratio 12%. Heating whole faba beans plus added hulls increased weight gain by 31% and decreased feed:gain ratio by 25%. This indicates that the hulls contain a potent inhibitor that is responsible for approximately one half (8/15) of the total growth inhibitors of faba beans would, therefore, be associated with the protein fraction.

Autoclave treatment did not affect the gelatinization of starch but completely inactivated the trypsin inhibitor and hemagglutinins.

In the second experiment a direct comparison of

TABLE 11. The effect of heat treatment and hull content on growth of broiler chicks.[1]

Diets	Weight Gain[2]	Feed Intake[2]	Feed: Gain[2]
	g	g	g/g
Raw-dehulled faba beans	436±8	800±11	1.84±0.02
Raw-whole faba beans	397±4	869±9	2.19±0.02
Raw-whole faba beans plus raw faba bean hulls	345±4	943±3	2.74±0.04
Autoclaved-dehulled faba beans	470±6	793±6	1.69±0.01
Autoclaved-whole faba beans	458±8	890±11	1.93±0.04
Autoclaved-whole faba beans plus autoclaved faba bean hulls	453±12	930±17	2.07±0.05

[1]There were 10 replicates of each diet with 5 birds per replicate. The experiment was initiated when the birds were 4 days of age and continued for 29 days. Average initial bird weight was 67±0.8 SEM.

[2]Means ± SEM.

SUMMARY OF ANALYSIS OF VARIANCE

Source	df	Weight Gain		Feed Intake		Feed:Gain	
		Mean Square	F	Mean Square	F	Mean Square	F
Level of hul	2	14,573	24**	99753	82.9**	2.07	194**
Heat treatment	1	68,276	112**	2	N.S	1.87	175**
Level of hull x heat treatment	2	6,931	11**	1695	N.S	0.37	35**
Error	54	607		1204		0.011	

**Significant at $P < 0.01$.

heating the hull or protein on a equal weight basis was made. The basal diet is shown in Materials and Methods (Table 2). Four combinations of raw and/or autoclaved faba bean hulls and protein concentrate were incorporated into four diets (Table 12). In this experiment heating the protein had no effect on weight gain. This indicates that heating the protein fraction has little effect on Leghorn chick growth if the protein content of the diet is already more than adequate to support growth. A improvement in weight gain probably would have been seen if the protein content of the diet was inadequate or if broiler chicks were used. There was a 3% improvement in feed:gain ratio upon heating the protein indicating a slightly improved utilization of the protein fraction of the faba bean upon heating. Heating the hull fraction improved weight gain an average of 12% and feed: gain ratio 8%. Heating both hulls and protein had no significant effect on weight gain over that obtained with heating of the hull fraction alone but the feed:gain ratio was improved by an additional 3% ($P<0.05$). Trypsin inhibitor and hemagglutinin activity were zero in the heated protein diets, and higher in the raw protein diet. The hull inhibitor does not seem to be a trypsin inhibitor, hemagglutinin or other protein since these are denatured with autoclaving after 20 minutes, whereas maximum chick growth response is not obtained until 30 to 40 minutes of heating. Also, there is no hemagglutinin activity in the hull (49). The inhibitor is not vicine, since heating does not affect it,

TABLE 12. Effect of autoclaving of faba bean protein concentrate and faba bean hulls on growth response in Leghorn chicks.[1]

Treatment		Trial 1		Trial 2
Hulls	Protein conc.	Weight Gain	Feed: Gain Ratio	Weight Gain
		g	g/g	g
Raw	Raw	64^{B2}	2.33^{Aa}	123^{B}
Raw	Autoclaved	66^{B}	2.26^{Ab}	124^{B}
Autoclaved	Raw	72^{A}	2.15^{Bc}	136^{A}
Autoclaved	Autoclaved	73	2.09^{Bd}	137^{A}
SEM.		0.8	0.02	1.2

[1]There were 4 replicates of each diet in Trial 1, and 12 replicates in Trial 2 with 10 birds per replicate in each trial. The trials were initiated when the birds were 5 days of age (Trial 1 and Trial 2) and were continued for 9 days (Trial 1) and 16 days (Trial 2). Average initial bird weight was 45.1 ± 0.2 g SEM.

[2]Means for each response criteria not sharing a common superscript letter within a column were significantly different at $P < 0.01$ (upper case) or $P < 0.05$ (lower case).

(Table 9) and as indicated previously, the starch fraction shows no growth inhibition when fed to growing chicks. Since the major growth inhibiting factor is associated with the hull fraction, further experiments on the hull fraction of the bean were initiated in an attempt to isolate and identify this compound.

III. Isolation of the Growth Inhibiting Factor From the Faba Bean Hull

Two experiments were conducted to determine if the growth inhibiting compound was concentrated in the water soluble or insoluble fraction of the hull. The objective of the first experiment was to determine if the factor causing poor utilization of faba beans could be extracted from the hull with water and to establish the influence of heat treatment on the raw and water extracted hulls. Four combinations of raw and autoclaved faba bean hulls, either non-extracted or extracted were incorporated into four diets (Table 4). The results (Table 13) demonstrate that both water extraction of hulls and autoclave treatment of hulls has a marked influence on the nutritional properties of the diets. Chicks fed autoclaved or extracted hulls as compared to those fed diets containing raw or non-extracted hulls had 24% greater weight gains and 5% and 3% improved feed:gain ratios, respectively. There was also an interaction ($P < 0.01$) between extracting and autoclave treatment with a maximum improvement in weight gain of 25% occurring with chicks fed diets containing heated extracted

TABLE 13. Comparison of growth and nutrient retention of Leghorn chicks when fed diets containing raw, autoclaved, and water extracted faba bean hulls.[1]

Diets	Weight Gain	Feed: Gain	Nutrient Retention[2]			
			Dry Matter	Protein (Nx6.25)	Fat (ether extract)	Crude Fiber
	g	g/g	%	%	%	%
Raw hulls	89±3	2.67±0.04	41±0.7	46±0.3	89±0.3	-6±1.7
Autoclaved hulls	110±2	2.54±0.02	49±0.0	55±0.6	91±0.0	11±0.8
Extracted hulls	110±1	2.60±0.01	49±0.3	53±0.3	90±0.3	15±1.7
Extracted autoclaved hulls	115±1	2.54±0.03	50±0.3	55±1.2	89±0.9	12±0.6

[1]There were 7 replicates of each diet with 10 birds per replicate. The test was initiated when the birds were 3 days of age and terminated after 15 days. Initial average bird weight was 41.5±0.1 SEM. Values are means ± SEM. [2]Fecal material from 3 of the 7 replicates of each diet was used to establish nutrient retention values. Chemical analyses were carried out on each replicate sample.

SUMMARY OF ANALYSIS OF VARIANCE

Source	df	Weight Gain		Feed:Gain		Percent Nutrient Retention Dry Matter		Protein		Fat		Crude Fiber	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Water Extraction	1	1157	46**	0.0112	NS	65	131**	33	24**	2.08	NS	339	69**
Heat treatment	1	1126	44	0.0680	17	56	112	108	76	0.75	NS	131	26**
Heat treatment x water extraction	1	438	17**	0.0082	NS	33	67**	33	24**	2.08	NS	312	63**
Error	8	25		0.0040		0.50		1.4		0.75		4.94	

**Significant at $P < 0.01$

hulls as compared to those fed diets containing raw, non-extracted hulls. Feed utilization improved a maximum of 5%. The results would suggest that either heat treatment or water extraction of the hulls were equally effective in eliminating the growth depressing factor and that each treatment singly was almost as effective as combined water extraction and autoclave treatment.

Nutrient retention (Table 13) increased similarily when faba bean hulls were either autoclaved, extracted with water or subjected to both treatments. The average percent increase in nutrient retention in birds fed the three diets containing processed hulls as compared to those fed raw hulls were: 20% for dry matter, 17% for protein (N x 6.25), 1% for fat and 300% for fibre. There was also an interaction ($P < 0.01$) in nutrient retention between extracting and autoclave treatments.

The trypsin inhibitor and hemagglutinin activities of faba bean hulls were either low or zero.

In the second experiment the water extract from the faba bean hulls were concentrated, lyophilized and fed to Leghorn chicks. Two levels of the extract either raw or autoclaved were incorporated into basal corn-soybean diets and were fed along with two control diets (Table 5, 14). Feeding the raw extract at 4.8% of the diet significantly ($P < 0.01$) reduced weight gain (86%) and feed:gain ratio (35%) in chicks compared to those fed the control diets

(Table 14). Autoclaving the extract improved weight gain and feed:gain ratio to the same values as the controls. The corresponding decrease in weight gain and feed:gain ratio for chicks fed the diets containing 2.4% extract as compared to the control diet was 18 and 8% respectively. Liver weight decreased 19% at the 4.8% extract level but not at the lower level. Pancreas weights in chicks fed the various diets were not significantly different ($P > 0.05$). The weight gain of the chicks fed the two control diets was the same. The feed:gain ratio of the chicks fed the diets containing 4.8% added cellulose was 5% greater ($P < 0.05$) than for chicks fed the control diet containing 4.5% added sucrose.

IV. Identification of the Growth Inhibitor

It was hypothesized that the component present in the hulls which caused the poor utilization of the faba bean could be a compound known as tannins. The term "tannins" covers a large variety of compounds but they can generally be divided into at least two large groups (Figure 1), the hydrolyzable tannins; which are readily hydrolyzed chemically or enzymatically and the condensed tannins which are not readily hydrolyzed and when exposed to acid conditions or hydrolysis from "phlobaphines", compounds with a deep reddish color. Hydrolysis of the faba bean water extract at 121° in 6N HCl produced a deep red compound. A number of compounds

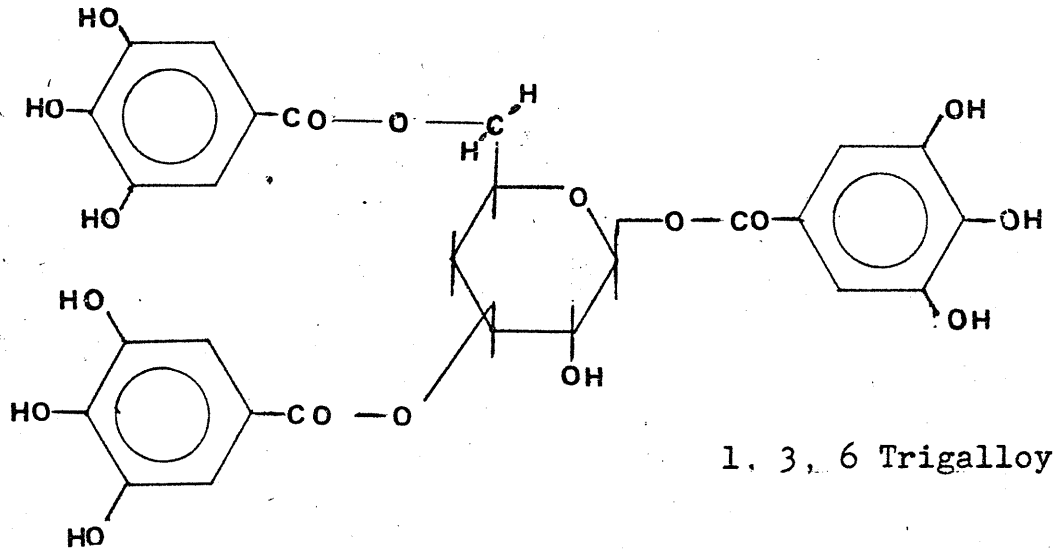
TABLE 14. Chick growth[1] as affected by feeding raw or autoclaved water-extracts of faba bean hulls.

Additions to basal diet	Weight[2] Gain	Feed:Gain[2,3] Ratio	Liver[2] Weight	Pancreas[2] Weight
	g	g/g	g/kg	g/kg
Sucrose	78 (100)[a3]	1.74 (100)[b]	32[a]	5.4
Cellulose	79 (101)[a]	1.82 (105)[b]	32[a]	5.7
Raw extract of hulls (4.8%)	42 (54)[c]	2.35 (135)[a]	27[b]	5.8
Autoclaved extract of hulls (4.8%)	77 (99)[a]	1.82 (105)[b]	31[a]	6.0
Raw extract of hulls (2.4%)	66 (85)[a]	1.88 (108)[b]	30[a]	6.2
Autoclaved extract of hulls 2.4%)	79 (101)[a]	1.79 (103)[b]	30[a]	6.2
SEM	2	0.03	1	0.2

[1]There were 5 replicates of each diet with 10 birds per replicate. The experiment was initiated when the birds were 3 days of age and lasted for 12 days. Initial average bird weight was 37.0±0.1 SEM. At the termination of the experiment the liver and pancreas of 2 birds per pen were immediately excised and dissected free of fat and other material and weighed. [2]Number in brackets represent values expressed as a percent of the group receiving the basal diet plus added sucrose. [3]Means not sharing a common superscript letter within a column were significantly different at $P < 0.01$.

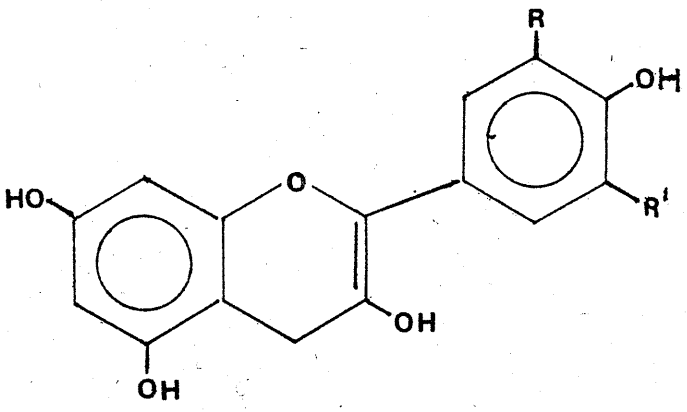
FIGURE 1.

Typical hydrolyzable tannin

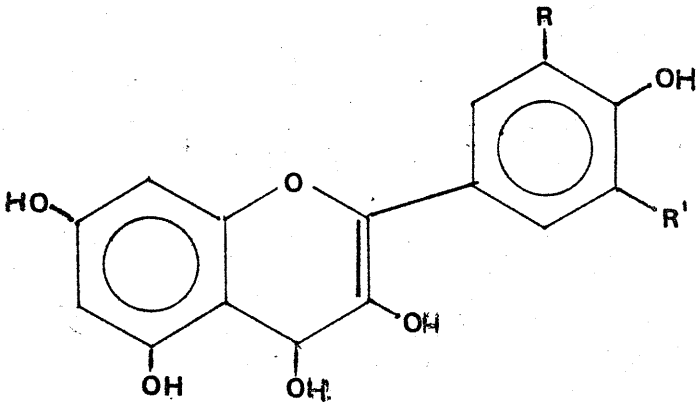


1, 3, 6 Trigalloy glucose

Typical condensed tannin sub-components



Flavan-3-ols



Flavan-3, 4 diols

are known to bind tannins. Some of these compounds are nylon, PVP (polyvinylpyrolidone), polyethyleneglycols, and keratin (43). A number of compounds commonly used in chromatographic separation were shown in preliminary experiments to be unsuitable for the separation of the tannins since a dark oxidized form of the tannin would bind irreversibly to the column resins thereby preventing any further purification of the growth inhibiting substance.

Studies of the Effect of PVP on the Growth Inhibitor in the Faba Bean Hull Extract

Two experiments were conducted; a chick feeding trial and a chromatographic separation to determine if PVP a compound known to bind tannins (43) could be utilized for purification of the growth inhibitor. In the growth trial (Table 15) the objective was to determine if the growth depressing activity of the water extract of faba beans could be altered or eliminated by the addition to the diet of PVP and to compare the response from this treatment with that obtained by autoclaving the water extract. The preparation of the water extract is described in Materials and Methods. The basal diet is shown in Table 3 of Materials and Methods. PVP addition to the basal diet and heat treatment improved chick performance and there was an interaction between level of PVP and type of extract (raw or heated) added to the diet for weight gain ($P < 0.01$) and feed:gain ratio ($P < 0.01$). PVP when added to the basal diet,

TABLE 15. Chick performance when fed diets containing raw or heated faba bean hull extract with and without PVP (polyvinylpyrolidone).[1]

Treatment (additions to basal diets)[2]	Tannin Levels in Diets	Chick Performance			
		Weight Gain	Feed: Gain Ratio	Liver Weight	Pancreas Weight
	%	g	g/g	g/kg	g/kg
6% raw extract + 2% starch	1.7±0.2	4	7.2	30	6.0
6% raw extract + 1.5% PVP + 0.5% starch	0.5±0.1	13	3.2	30	6.3
6% heated extract + 2% starch	0	24	2.2	27	6.7
6% heated extract + 1.5% PVP + 0.5% starch	0	36	1.8	31	7.2
6.5% starch + 1.5% PVP (control)	0	39	1.8	34	7.3
8% starch (control)	0	38	1.7	33	6.8
SEM		1	0.1	0.5	0.2

[1]Initial average bird weight was 44.6±0.1 g SEM. The number of birds per pen was 5, with 4 pens per treatment. [2]See Materials and Methods and Table 3 for data on preparation of diets, faba bean hull extracts and assay procedures. The percent protein (Nx6.25) ± SEM of the diets containing raw extract, raw extract plus PVP, heated extract, heated extract + PVP, PVP, and no additions were: 21.4±1; 22.0±0.1; 22.1±0.2; 21.3-0.1 and 21.4±0.1 respectively. The proximate analyses of other ingredients were altered in proportion to amount of PVP, starch or faba bean extract added to the diet. The average percent proximate analysis ± SEM of the raw extract were: dry matter, 92.6±0.3; protein (Nx6.25), 9.8±0.1; fat (ether extract), 0.26±0.1; crude fibre, 0.0; and ash 15.7±0.1 respectively. All values represent average ± SEM of duplicate analyses.

Cont'd.....

TABLE 15 cont'd.

SUMMARY OF ANALYSIS OF VARIANCE

Source	df	Weight Gain		Feed: Gain		Liver Weight		Pancreas Weight	
		Mean Square	F	Mean Square	F	Mean Square	F	Mean Square	F
Heat treatment	2	47069	302**	30	117**	53	18**	2	4*
PVP level	1	6240	40**	12	46**	10	3	1	2
Interaction	2	2491	16	10	39	11	3	.03	.06
Error	18	156		0.25		3			

**P < 0.01

*P < 0.05

overcame 26% of the growth inhibition of the raw extract while autoclave treatment of the extract overcame 54% of the growth inhibition. A combination of heat treatment and PVP additions to the basal diet, which provided maximum improvements, overcame 92% of the growth depressing effect of the raw extract when compared to the control diet. PVP alone in a control diet did not affect weight gain. The pattern of response in feed:gain ratio for chicks fed autoclaved as compared to those fed the raw extract or the diets with PVP additions was similar to that observed for weight gain. Liver size was reduced ($P < 0.01$ when chicks were fed an autoclaved extract as compared to a raw extract but was not affected by PVP additions to the basal diet. Pancreas weight was not affected by any of the treatments ($P > 0.05$).

Trypsin inhibitor and hemagglutinin activities in the raw and autoclaved extracts were zero.

In the second experiment a large column was packed with insoluble PVP. A water extract of faba bean hulls was separated into two fractions; a soluble fraction which could be removed after prolonged elution with water and a bound fraction which could only be removed from the PVP by eluting with 0.2N NaOH. Although this technique fractionated the water extract it was abandoned since the high pH needed to remove the bound fraction resulted in the production of chemically modified compounds. The procedure did however

demonstrate that PVP could effectively bind all of the tannin components from a water extract of faba bean hulls.

V. Concentration of the Growth Inhibitor

Acetone-Water Fractionation of the Faba Bean Hull Water Extract

The lyophilized water extract of faba bean hulls was partially purified following acetone fractionation. Four acetone fractions were prepared as described in Materials and Methods. They were: (1) a fraction insoluble in water. The components of this fraction constituted material that was originally soluble in water (during the original extraction procedure) but rendered insoluble in the drying process; (2) a fraction that was soluble in water but insoluble in 75:25 acetone-water solution; (3) a fraction soluble in 75:25 acetone-water solution but insoluble in 90:10 acetone-water solution, and (4) a fraction soluble in a 90:10 acetone-water solution. Each fraction was incorporated into a basal diet (Table 1, Materials and Methods) in proportion to the amounts isolated from the water extract (Table 16). The results demonstrate that nearly all of the growth inhibition was associated with the diets containing the unfractionated water extract and the 90:10 acetone-water supernatant fraction. The above two diets were the only diets that contained a significant amount of condensed tannins. Feed:gain ratio was affected in a manner similar to that observed for weight gain.

TABLE 16. Growth depressing effects of certain acetone-water fractions prepared from a water extract of faba bean hulls when fed to chicks[1]

Additions to basal diet[2]	Level of added Fractions	Chick Performance: Dietary Tannins	Chick Performance: Weight Gain	Chick Performance: Feed:Gain Ratio
	%	%	g	g/g
Control diet (basal + 8% starch)	0	0±0.0	40[a3]	1.8[c]
Water extract	7	2.4±0.2	1[c]	30.7[b]
Insoluble precipitate	0.8	0.1±0.0	39[a]	1.8[c]
Acetone, 75% precipitate	2.1	0.0±0.0	39[a]	1.8[c]
Acetone, 75-90% precipitate	1.5	0.02±0.0	34[b]	1.9[c]
Acetone, 90% supernatant	4.3	2.8±0.2	4[c]	11.4[a]
SEM	--	--	1	2

[1]A complete randomized design involving 6 treatments with 4 replicates of birds each was employed. The experiment was initiated when the birds were 4 days of age and was terminated 6 days later.

[2]The total additions to the basal diet (given above) was 8%. The percent protein (Nx6.25) ± SEM of the diets containing added starch only (control); water extract, insoluble precipitate, 75% acetone precipitate, 75-90% acetone precipitate, and 90% acetone supernatant were: 21.4±0.1, 22.1±0.2, 21.8±0.2, 21.5±0.1, 21.2±0.1 and 21.3±.2. The levels of other dietary components were not affected by the additions to the basal diet. The average percent values for all diets ± SEM were: dry matter 91.5±0.1; fat (ether extract), 6.6±0.1; crude fiber, 3.2±0.1; and ash, 7.0±0.2. See Materials and Methods for further details on preparation of diets, preparation of the different fractions from faba bean hulls, and assay procedures.

[3]Means for each value not sharing a common superscript were different at $P < 0.01$.

LH-20 Sephadex Chromatography of the Faba Bean Hull Extract

The objective of this experiment was to determine if the growth inhibitor in the 90:10 acetone-water soluble fraction prepared from a water extract of hulls could be further purified using LH-20 Sephadex chromatography. A fraction soluble in 90:10 acetone-water was prepared as in Materials and Methods. Upon applying this fraction to a Sephadex LH-20 column two major fractions were eluted, fraction A (low molecular weight polyphenolics) and fraction B (condensed tannins). These two fractions plus the material insoluble in 90:10 acetone-water plus the original acetone-water (90:10) soluble fraction (composed of A & B) were incroporated into basal diets (Table 1) and were fed to Leghorn male chicks. The levels of the various fractions added to the diets were proportional to the amounts isolated from the hulls (Table 17). Chicks fed the diets containing purified condensed tannins (fraction B) or the 90:10 acetone-water soluble material (concentrated tannins containing A and B) either lost weight or grew slowly. As a consequence, the efficiency of feed utilization was depressed when compared to that of birds fed the control diet. The performance of birds fed the basal diet that contained the insoluble acetone precipitate (which probably contained insoluble complexed condensed tannins) also had reduced weight gains (25%, $P < 0.01$) and feed:gain ratios (21%, $P < 0.01$) as compared to the control birds. Feed

TABLE 17. Growth depressing effects of certain fractions prepared from a water extract of faba bean hulls when fed to chicks.

Addition to basal diet[1,2]	Dietary Tannins	Chick Performance			Nutrient Retention				
		Feed Intake	Weight Gain	Feed: Gain	Dry Matter	Protein (Nx6.25)	Fat (ether extract)	Crude Fiber	Amino Acid
	%	g	g	g/g	%	%	%	%	%
Control diet (basal + 8% starch)	0.0±0.0[a]	68[a3]	36[a]	1.9[d]	67.7[a]	54.9[a]	64.6[c]	1.8[a]	84.6[a]
Acetone-water (90-10) precipitate (6%)	1.8±0.2	68[a]	27[b]	2.3[c]	56.6[c]	38.0[bc]	76.1[ab]	-15.6[bc]	73.6[b]
Fraction A (1.6%)	0.0±0.0	58[a]	30[b]	1.9[d]	62.4[b]	45.7[ab]	66.0[c]	-10.2[ab]	83.4[a]
Fraction B (3.9%)	3.9±0.2	21[b]	-6[c]	-5.0[a]	53.1[c]	8.3[d]	82.3[a]	-19.7[c]	61.0[c]
Acetone-water (90-10) soluble fraction (4.5%)	3.4±0.1	32[b]	4[c]	5.8[b]	54.3[c]	22.5[c]	84.5[a]	-29.7[c]	66.2[c]
SEM		3	1	0.8	1.2	2.6	2.9	3.4	2.0

Cont'd.....

Table 17 cont.

[1]See Materials and Methods and Table 3 for a description of the basal diet and the procedures followed in preparing the various fractions from faba bean hulls. The values in brackets in this table represent percent of the particular fraction added to the diet. The total amount of added fractions plus starch was 8%. The percent protein (N x 6.25) ± SEM of the diets containing 8% added starch, 6% acetone precipitate, 4% PVP eluate, 1.6% fraction A, 3.9% fraction B and 4.5% acetone-water soluble fraction were: 21.2±0.1, 22.1±0.1, 21.5±0.1, 21.9±0.1, 21.2±0.2, and 21.4±0.1 respectively. The average percent dry matter, fat (ether extract), crude fiber, and ash ± SEM for all remaining diets which were not greatly affected by diet were: 91.6±0.2, 6.8±0.2, 3.4±0.1 and 7.0±0.2 respectively. Initial average weight per bird was 47.8±0.1g SEM.

[2]There were 6 treatments with 4 replicates of 5 birds each. The experiment was initiated when the birds were 4 days old and was terminated 6 days later.

[3]Means for each response criteria not sharing a common superscript letter within columns were significantly different at $P < 0.01$.

intake in this case, however, was not affected ($P > 0.05$). Fraction A (from LH-20 column) included in a basal diet reduced feed intake values by 15% ($P < 0.05$) and weight gains by 16% ($P < 0.01$). Feed:gain ratios, in contrast were the same as the control group ($P > 0.05$).

Nutrient retention in chicks was also markedly influenced by the nature of the diet the chicks were fed (Table 17). The chicks fed the diets containing the condensed tannins as compared to those fed the control diet had decreased dry matter, protein (N x 6.25) and amino acid retention. The chicks fed the diet with 3.9% added fraction B (pure condensed tannin separated on the Sephadex LH-20 column) retained 22% less dry matter, 85% less protein (N x 6.25), and 27% less amino acids. In contrast, fat (ether extract) retention increased by 27%. Chicks fed the acetone insoluble form of condensed tannin, possibly a protein tannin complex, had dry matter, protein (N x 6.25)and amino acid retentions of 16, 31 and 13% less, and fat (ether extract) retention of 15% more than those obtained with the control birds. Amino acid, fat (crude fibre), protein (N x 6.25) and dry matter retention in chicks fed diets containing the low molecular weight components (fraction A) were either the same or only slightly lower than the values obtained for the birds fed the control diet. The crude fibre retentions were negative for all treatment groups except those fed the control diet. There were also differences

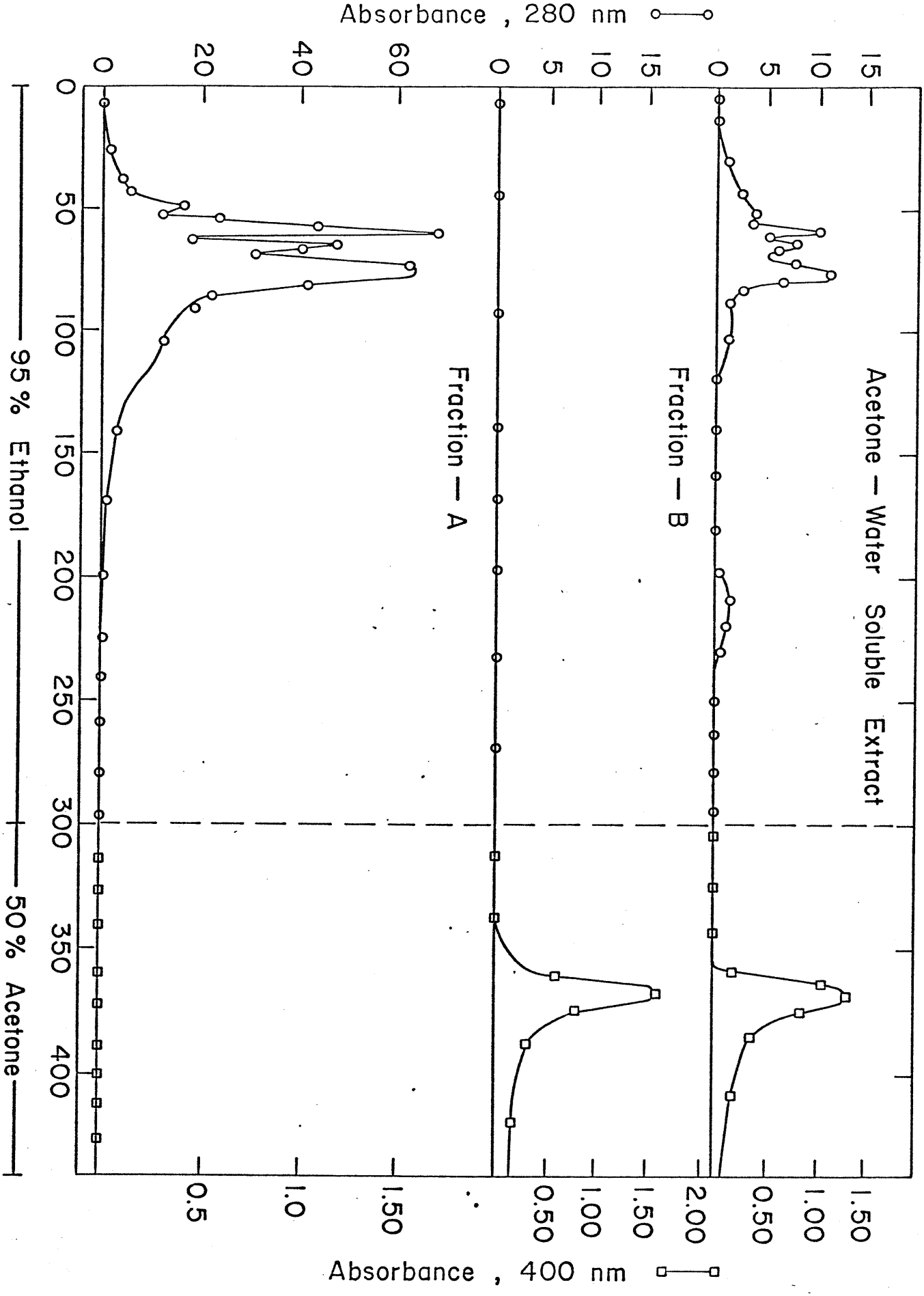
in the retention of individual amino acids ($P < 0.01$). The values, for all diets, ranged from a high of 85.1 and 84.2% for arginine and glutamic acid to a low of 75.6 and 65.2% respectively for proline and threonine. The average retention of lysine and methionine, two potentially limiting amino acids, were 79.2 and 77.9% respectively. There was no interaction between amino acid retention and diet at the 5% level of probability indicating that condensed tannins affected the availabilities of all amino acids to a similar degree.

VI. Properties of Faba Bean Condensed Tannins and Low Molecular Weight Polyphenolics

Analytical Resolution of Tannin and Non-Tannin Components

The large scale preparative isolation of condensed tannins which was outlined in Materials and Methods, yielded two distinct fractions, A and B. The effectiveness of the preparative column in resolving the low molecular weight components (fraction A) from the higher molecular weight tannin components (fraction B) was demonstrated when these fractions were subjected to rechromatography on an analytical scale (see Materials and Methods). A typical chromatogram for the separation on this latter column of the 90:10 acetone-water fraction, together with rechromatography of fractions A and B (Fig. 2) demonstrated that fractions A and B yielded only single peaks when rechromatographed and that the 90:10

FIGURE 2. Separation of tannins from non-tannins by absorption on Sephadex LH-20 and elution with 95% ethanol followed by 50% acetone.



acetone-water fraction could be resolved into fractions A and B. The relative amounts of components A and B, as estimated from absorbancy values and dry matter recoveries from the column were 25% and 75% respectively.

Chemical Characterization of Fraction A and B Eluted from the Sephadex LH-20 Column

In order to establish the nature of the components isolated from the column further chemical characterization was carried out. An absorption spectrum of the two components (Fig. 3) demonstrates that the two fractions had different spectras and absorption maxima in both acidic and basic solutions. A series of tests were conducted to establish the relative concentrations of condensed tannins in the two fractions. The results of these tests which are summarized in Table 18 demonstrated that fraction A did not contain any component that gave a positive condensed tannin test whereas fraction B did. These tests included precipitation of ferric chloride, precipitation of gelatin, inactivation of lactate dehydrogenase activity and a positive vanillin-hydrochloric acid test. In this latter test, the color intensity of fraction B was 3.5 fold greater than that of the reference standard, catechin (from Sigma). Fraction B which is of a similar degree of purity as the condensed tannin obtained by Strumeyer and Malin (66) from sorghum grain was therefore used as the new standard and assigned a value of being 100% condensed tannins.

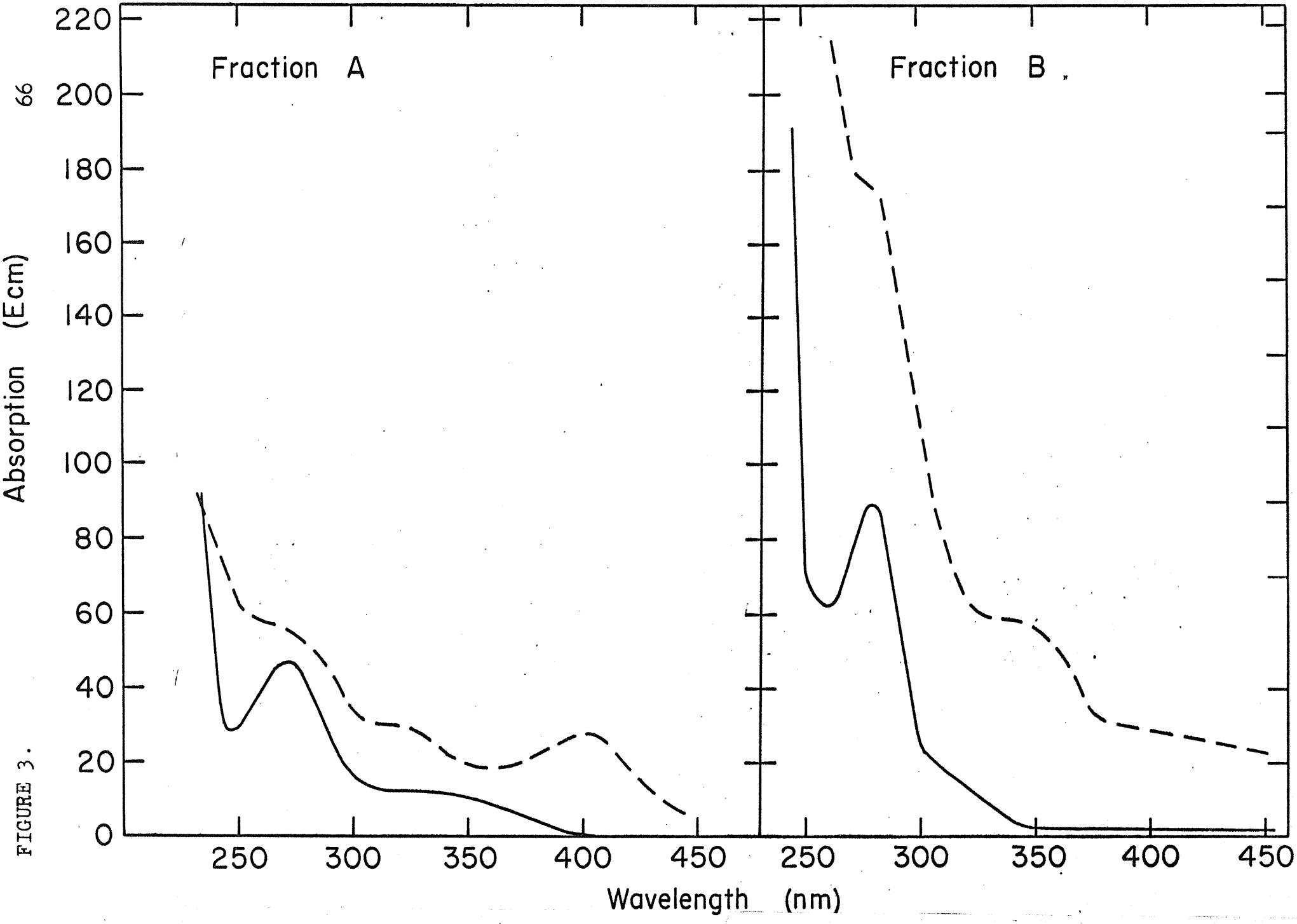

Fraction A
Fraction B
Absorption (Ecm)
220
200
180
160
140
120
100
80
60
40
20
0
250
300
350
400
450
Wavelength (nm)

FIGURE 3.

TABLE 18. Properties of fraction A and B.

Property or Test	Fraction A	Fraction B
Solubility: Soluble	H_2O	H_2O
	alcohol	alcohol
	-	acetone
Insoluble	-	ether
Amino acid content	3.0%	0.1%
Ferric chloride in H_2O	green solution	black precipitate
Gelatin	no precipitate	precipitated
Activity of lactate dehydrogenase	no effect	inhibition
Absorption (maxima of distinct peaks:		
in basic or neutral solutions	405 nm	350 nm
in acidic solutions	272 nm	278 nm
Color of aqueous solutions		
Acidic solution	pale yellow	pale tan
Basic solution	deep yellow	reddish brown
Reversibility of color change with pH	reversible	reversible
Two dimensional paper chromatography	discreate spots	indiscreate band
Condensed tannin level (vanillin - H Cl test)	0%	100%

The concentration of total amino acid in fraction A was 3.0% of the total sample. The principle amino acid was dihydroxyphenyl alanine, 1.2%. Fraction B contained only a trace of amino acids (0.02%) and gave only a slightly positive ninhydrin test.

Thin Layer Chromatography of the Faba Bean Hull Fractions

Two dimensional thin layer chromatography of the three fractions was also carried out (Fig. 4, 5, 6). A chromatogram of the 90:10 acetone-water soluble fraction of the water extract of faba bean hulls is shown in Figure 4. This chromatogram shows simpler phenolic compounds as unshaded areas and tannin-like substances in the cross-thatched areas. The fact that chromatography on Sephadex LH-20 had accomplished a clean separation between simpler phenolics and tannins is demonstrated by Figure 5 which shows the simpler phenolic compounds and Figure 6 which shows tannins. The individual components in fraction A have not been identified except that two or three of the spots may be phenolic amino acids, including the amino acid, dihydroxyphenyl alanine. Preliminary studies on the nature and chemical structure of the condensed tannins (fraction B) has indicated that two of the subunits are delphinidin and cyanidin. Tannic acid

FIGURE 4

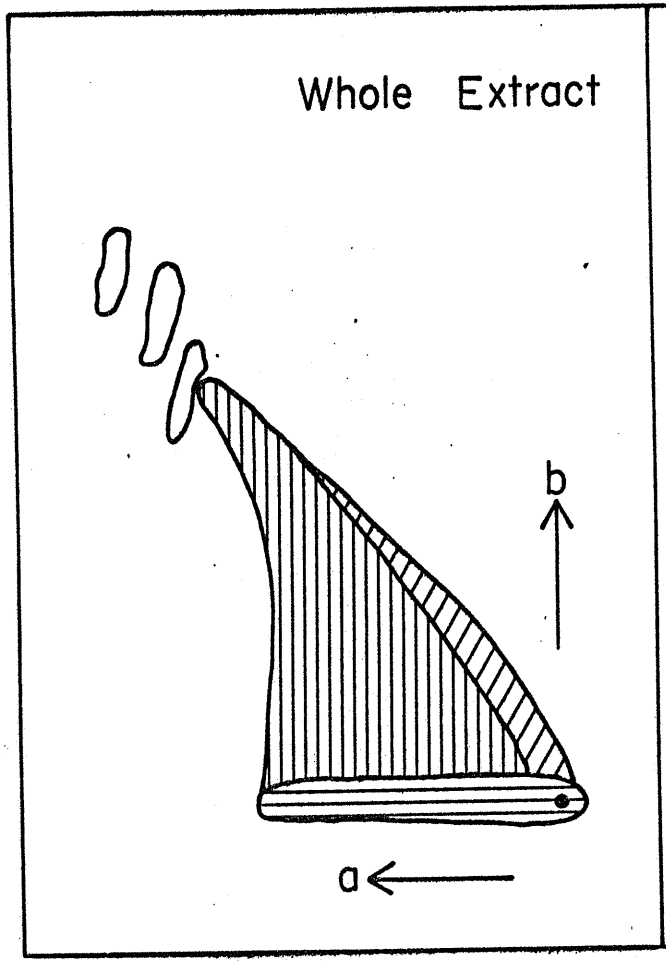


FIGURE 5

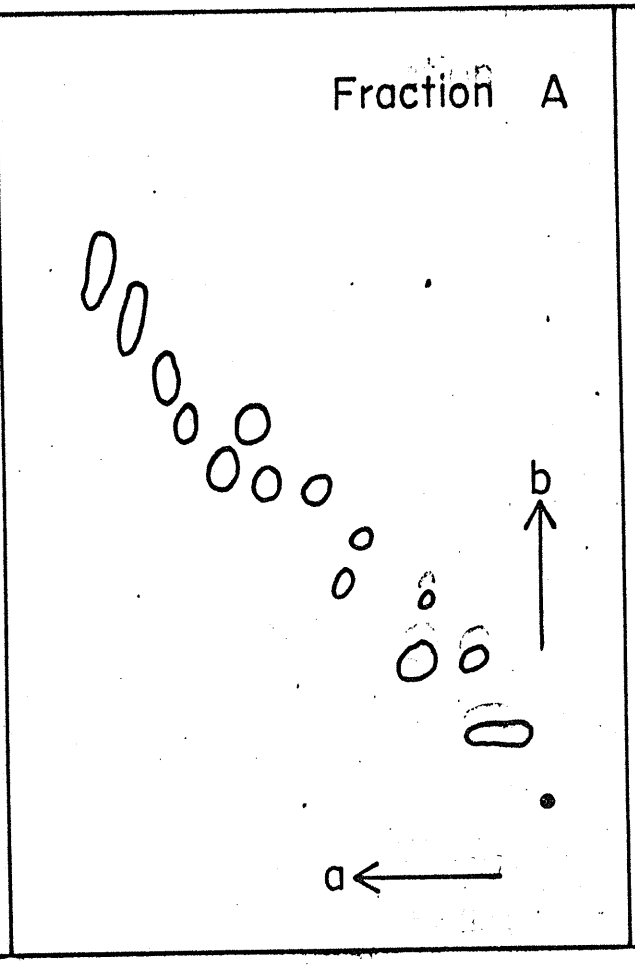
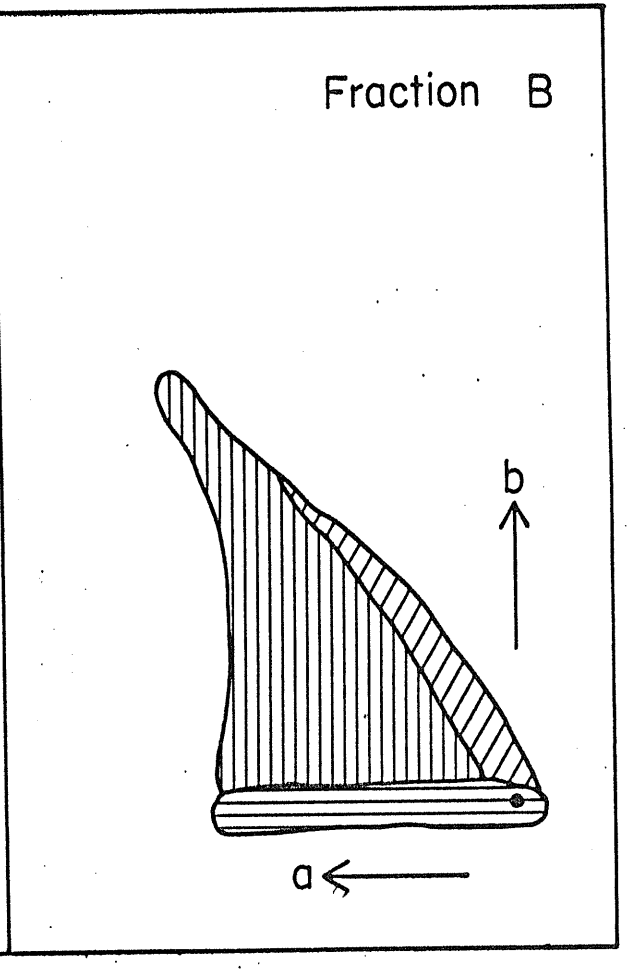


FIGURE 6



TWO DIMENSIONAL CHROMATOGRAPHY OF DIFFERENT FRACTIONS PREPARED FROM THE TESTA OF DIANA FABABEANS.

(gallic acid) a constituent of the hydroyzable tannins (43) was not identified in any of the faba bean hull fractions or in acid hydrolysates thereof. The amino acid composition of fraction A and B, along with the fraction soluble and insoluble in 90:10 acetone-water are shown in Table 19. Fraction A contains most of the amino acid with only trace amounts, except DOPA in fraction B. Most of the amino acids precipitated out of solution with the 90:10 water-acetone insoluble fraction.

VII. Cultivar Comparison

Although different local cultivars demonstrated almost identical tannin levels (Table 20), it was suspected that tannin levels were affected by cultivar differences, growing conditions and the age and storage condition of the bean. Harris et al (28) have shown that bagging of the panicles at mid-anthesis reduced tannin content and the brown color, and significantly increased dry matter digestibility. The effect of age on the measurable tannins in the bean is shown in Table 21. Analyzing three cultivars stored over three years from the same location demonstrated that tannin levels decreased significantly upon storage. The average decrease over a two year period was 39% as measured by the vanillin - hydrochloric acid method (10) of Burns. The maximum decrease of the varieties tested was 46% for the cultivar Hertz-Freya. In another study condensed

TABLE 19. Amino acid analysis of fraction A and B from Sephadex LH-20 column and 90% acetone soluble and insoluble fractions.

Amino Acid	Fraction A	Fraction B	90% Soluble	90% Insoluble
Lysine	.023	0.004	.007	0.106
Histidine	.004	0.001	.002	0.045
Ammonia	0.768	0.019	V.High	0.565
Arginine	0.218	0.003	0.049	0.816
Aspartic acid	0.252	.009	0.118	0.055
Threonine	0.163	.002	0.042	0.125
Serine	0.049	.003	0.019	0.164
Glutamic acid	0.098	.009	0.041	1.330
Proline	0.106	--	0.032	0.239
Glycine	0.108	.008	0.030	0.355
Alanine	0.161	.002	0.032	0.287
Cystine	--	--	--	--
Valine	0.179	.002	0.042	0.112
Methionine	0.020	--	0.006	0.015
Isoleucine	0.207	.003	0.057	0.068
Leucine	0.062	.002	0.016	0.050
Tyrosine	0.080	.003	0.021	0.051
Phenylaanine	0.130	.003	0.032	0.039
DDPA	1.165	.021	0.502	2.255
TOTAL (without ammonia)	3.025	.075	1.048	6.112

Results in % A.A.

TABLE 20. Condensed tannin levels of faba bean cultivars grown in the same year and location.[1]

Cultivar	Condensed tannins in hull ± SEM.[2]
	(%)
Ackerperle	4.7±0.1
Bell	4.3±0.1
Blue Rock	4.3±0.1
Diana	4.6±0.1
Erfordia	4.1±0.1
Foecnevyje	4.0±0.1
Herra	4.2±0.1
Kleinkornige	4.2±0.1
Wales	4.2±0.1
Average	4.3±0.2

[1]Analysis of each cultivar represent the average value obtained from each of six separate locations in Manitoba and Saskatchewan. The faba beans were grown in the summer of 1972.

[2]Mean ± SEM of quadruplicate samples from each of the 6 locations.

TABLE 21. Effect of age of faba beans on condensed tannin levels in the hulls.

Cultivar	Year Grown	Location	Percent Hull	Percent Tannin in Hull[1]
Diana	1973	Manitoba	13.8±0.1	1.79±0.1
	1974	Manitoba	13.4±0.4	2.97±0.2
	1975	Manitoba	12.6±0.2	3.34±0.1
Diana	1974	Fredricton	12.5±0.3	2.06±0.1
	1975	Fredricton	12.2±0	3.40±0.4
Hertz-freya	1974	Fredricton	13.6±0.2	1.90±0.1
	1975	Fredricton	13.3±0.1	3.54±0.1
Ackerperle	1974	Fredricton	13.5±0.2	2.89±0.1
	1975	Fredricton	12.1±0.3	4.20±0.5

[1]Mean ± SE of quadruplicate testa and duplicate condensed tannin values.

tannin levels in several cultivars of faba beans were compared (Table 22). The varieties Kodrim, Fidrim and Triple White were all found to contain no measurable tannins. The seed coats of these three varieties were all white.

TABLE 22. Comparative properties of faba beans and faba bean hulls.

Cultivar	Color	Bean Size	Percent Hull[1]	Percent Protein[1]	Color of Hull Extract in Water	Condensed Tannin Level in Hull
		(g)				(%)
Hertz-freya	brown	0.4±0.01	14±0.3	27±0.1	dark	6.0±0.1
Diana	brown	0.3±0.03	13±0.3	26±0.1	dark	4.4±0.2
Small Pod	brown	0.6±0.01	15±0.3	27±0.1	dark	3.8±0.2
Kondrim	white	0.9±0.04	13±0.3	25±0.1	colorless	0
Triple White	white	1.1±0.03	13±0.4	28±0.1	colorless	0
Fidrim	white	1.1±0.03	13±0.2	27±0.1	colorless	0
Regina blanca	tan	1.0±0.01	14±0.1	--	dark	2.0±0.2
Exhibition Long Pod	brown	1.4±0.14	15±0.2	29±0.1	dark	2.3±0.1
Brown Windsor White	white	1.4±0.09	14±0.3	27±0.1	dark	2.4±0.1

[1]Mean ± SEM. All analyses were in quadruplicate except for protein analysis which was in duplicate.

DISCUSSION

The advantages of faba beans as a protein crop are impressive. They are high yielding and well adapted to the moister cereal growing areas of Canada (23). The beans are frost tolerant in spring and grow rapidly even under cool conditions. They grow approximately 1 meter in height and have excellent straw strength permitting straight combining. They exceed the cereal crops in protein content and are much higher in the essential amino acid lysine. They have relatively good metabolizable energy values and they can be readily dehulled which increases both metabolizable energy and protein content. The hull byproduct can be fed as a roughage to ruminant animals with little deleterious effect (20).

Faba beans though high in protein are low in the sulfur amino acids. They are also low in metabolizable energy compared to cereal crops and have anti-nutritional factor (s) which decreased their utilization. The nutritional quality of faba beans, however, can be markedly improved by processing and by supplementing faba bean containing diets with methionine. The requirement for methionine may be heightened due to the inhibiting components in faba beans. If the action of tannins in the intestinal tract of chickens is similar to growth inhibitors in soybeans

it is likely that raw faba beans caused an increased secretion of proteolytic enzymes into the intestinal tract which would cause a increased demand for methionine for the production of pancreatic enzymes. Methionine depletion due to its use as a methyl donor for the detoxification of the metabolites of tannic acid, pyrocatechol and pyrogallol, could also increase the demand for methionine if tannic acid or similar hydrolyzable tannins were present in the feed (56). Tannic acid yields gallic acid and glucose upon hydrolysis, neither of which were found upon hydrolysis of extracted faba bean tannins followed by separation on chromatographic paper. Many authors use tannic acid as a model for all tannins and compare directly the results of feeding a high tannin grain or legume with feeding tannic acid. Since the grain or legume may not contain hydrolyzable tanninns (like tannic acid) but like faba beans contain condensed tannins, this kind of comparison is very limited and misleading in its interpetation.

Heating the faba beans before they are mixed into chick diets enhances their utilization. The effect is quite dependent on the method of heating and particularily on the moisture levels. Autoclaving and extruding are the most effective heating processes for improving the nutritional quality of faba beans. It has been shown that the effect of heating is not due to increased digestibility of the starch component due to its gelatinization. Autoclaving and extruding the bean before being mixed into a chick diet give

similar chick growth rates yet only extruding increases the degree that starch is gelatinized. Heating (autoclaving 121°) has a more pronounced effect on the utilization of faba beans as the hull content increases. Yet the dehulled bean shows a significant improvement in quality upon heating. It, therefore, seems that at least one-half of the improved nutritional quality of the faba bean for growing chicks is associated with the destruction of factors in the cotyledon portion of the bean. Within the cotyledon it would appear that the growth inhibiting factor is concentrated in the protein as compared to the starch fraction. The remaining one half of the total growth inhibiting potential of faba beans is associated with the hull portion of the bean, a fraction which comprises approximately 13% of the total bean.

The biological utilization of protein depends upon factors such as protein content, protein quality and protein digestibility. These factors are influenced by amino acid content, the presence of enzyme resistant peptides and enzyme-inhibiting substances, and the structural features and amino acid sequence of proteins (35). Faba bean protein quantity and quality seem to be adequate for growing chicks if the diets are supplemented with the essential amino acid methionine. It, therefore, seems likely that the improved nutritive value obtained with feeding heated dehulled faba beans is due to improved protein digestibility. This improved digestibility may be due to a increased amino acid availability. Kakade (35) states that "protein hydrolysis

is considerably affected by its tertiary structure". Any change in this structure through denaturing agents such as heat which would expose enzyme-susceptible bonds would result in an increased rate of protein hydrolysis. Faba beans and soybeans react similarily to heating in that the protein retention is increased probably due to increased digestibility. Studies done on soybean digestibility may be relevant to faba bean digestibility. It has been shown (69) with soybeans that heating improves the digestibility 20%, and this increase takes place beyond the duodenum. Using ^{14}C labelled casein (68) it has been shown that most of the difference in digestion occurs in the jejunum and ileum and the undigested protein is of dietary origin. Glick (26-27) on the other hand, feeding tannic acid to chicks and using ^{14}C labelled casein, reported that most of the increased protein excreted was of endogenous origin. This study was not extensive. There was much variability in the results, therefore the conclusions may not be valid. Also the authors employed tannic acid as a standard rather than condensed tannins.

The presence of known growth inhibiting substances in the faba bean protein has been shown (48, 49, 78). It is known that the cotyledon contains trypsin inhibiting compounds and hemagglutinins. Trypsin inhibitors inhibit trypsin production causing a increased secretion of trypsin as well as decreased protein digestion. Hemagglutinins are substances which are thought to combine with the gut wall

lining and alter the absorption of certain components across the cell walls. Since it has been shown that maximum growth in chicks occurs after 30 minutes of autoclaving and these components are completely inactivated after 10 and 20 minutes of autoclaving it seems unlikely that they are responsible for any growth inhibition in the protein fraction of the faba bean. The protein portion of the bean may also contain tannin components whose inactivation upon heating is resposible for the improved nutritional quality of the protein. This is difficult to determine since the protein interferes with most tannin determinations.

The positive effect upon chick growth with heating of faba bean protein isolate before being added to a diet seems to be quite dependent on protein level in the diet and on the breed of the experimental chick. Heating the protein did not increase growth and only improved feed:gain ratio 3% when the chicks used in the experiment were male Leghorns and the protein levels were above adequate. Much larger differences in weight gain and feed efficiency were observed when broiler chicks were fed heated dehulled bean as compared to raw dehulled bean. These differences were much greater, probably since broiler chicks were used and protein levels were not excessive. Since broiler chicks grow at approximately twice the rate of Leghorns in the first weeks of life, any improvement in rate of digestion of the protein would be magnified in regard to weight gain and to a less extent with

feed efficiency, with the broiler compared to the Leghorn chick.

Vicine, a compound known to be present in faba beans and possibly associated with favism in humans, is not destroyed when faba beans are autoclaved for 40 minutes or when the beans are extruded 152°. Therefore, vicine destruction is not a factor responsible for improved utilization of faba beans due to heat treatment.

Chicks fed dehulled as compared to whole faba beans have markedly improved weight gain and reduced feed:gain ratios. Increasing the level of hulls in the diet has the opposite effect. Heating the faba bean diets which had added faba bean hulls had a much greater improvement on chick growth and feed efficiency than heating a dehulled faba bean fraction. Extracting the hulls with water before being added to a basal diet gives similar results as heat treatment of the hulls in regard to chick growth response. A combination of extraction and heating, however, doesn't significantly improve chick growth response above that obtained with either treatment singly. The results of feeding trials with dried water extracts obtained from faba bean hulls were consistent with the above observations. The incorporation of the water extract into a basal chick diet resulted in dramatic decreases in weight gains and efficiency of feed utilization. The results suggested that the hull portion of faba beans, which accounts for approximately 50% of the total growth inhibiting potential in faba beans, contains a inhibitor that is water extractable,

heat labile and is bound by PVP. Heat treating the water extract before being added to the basal diet or the addition of PVP (polyvinylpyrolidone) to the diet decreases the growth retarding effect and a combination of the two eliminates most of the inhibition. Heat treatment of the extract doesn't overcome all the inhibiting effect. This is probably due to either insufficient protein in the extract for the binding of the tannin inhibitor or with a high concentration of inhibitor it takes longer for all the inhibitor to inactivate. Prolonged heating would have shown a greater effect on chick growth response. PVP is a compound which is known to bind condensed tannins (42). In an "in vitro" study using PVP as the packing material in a chromatography column all of the condensed tannins were removed from a water extract of the hulls. Therefore the reason that the PVP didn't completely alleviate the poor growth response upon feeding the water extract is probably due to the low level added in the diet.

Acetone fractionation was determined to be a good method for the concentration and purification of the tannins from the water extract of the faba bean hulls. Most of the growth inhibiting tannin components were soluble in 90% acetone, 10% water. The acetone fractionation procedure was particularily effective in that it not only increased the concentration of the inhibitor but also eliminated a readily oxidizable component from the extract which interfered with any subsequent purification steps. Elution on a Sephadex

LH-20 chromatography column similar to the method used by Struymeyer and Malin (66) separated the 90:10 acetone-water soluble fraction into two further fractions. The first peak eluted with 95% ethanol contained low molecular weight polyphenolic substance and some amino acids. The second peak eluted with 50% acetone contained relatively pure condensed tannins.

Upon feeding these fractions to Leghorn chicks in a basal diet it was found that most of the inhibition of growth was associated with the original water extract, the 90:10 acetone soluble fraction and the second fraction off the LH-20 fraction (fraction B). The first LH-20 fraction also depressed growth in chicks but to a much smaller degree. This effect may be associated with the astringent taste of this fraction since feed intake was reduced but there was no decrease in feed efficiency. This fraction may contain compounds which are similar to ethyl acetate soluble, astringent phenolic compounds reported to be present in carobs (69). Chicks fed the fraction not soluble in the 90:10 also had reduced weight gains. However, in contrast with the effects observed for fraction B, feed intake levels were the same as the control birds but efficiency of feed utilization was depressed. Fraction B, when incorporated into a diet and fed to chicks reduced both feed intake and feed efficiency which resulted in markedly reduced weight gains.

The decreased efficiencies of feed utilization are

the result of decreased nutrient retentions. Nutrient retentions were similar for fraction B and for the insoluble fraction (not soluble in 90:10 acetone-water but originally soluble in water) but differed from the non-tannin fraction (A). The insoluble fraction is thought to be made up of a tannin-protein complex which though insoluble in 90% acetone still is a reactive inhibitor, possibly due to the presence of free protein binding sites. The tannin containing fractions reduced dry matter, protein, amino acid and crude fibre (negative values) and elevated fat retention in chicks. The non-tannin fraction (A) had no effect on nutrient retention compared to a control diet. The negative fibre retention values obtained when tannins were incorporated into a basal diet indicate that chicks fed these diets were excreting a greater content of crude fibre than they were consuming. Studies on the nature of the fibre fraction in the excreta of birds fed a basal diet and a basal diet plus added tannins demonstrated that the portion of the lignin as compared to cellulose was much higher in those birds fed the latter diet. These observations and the fact that tannins will interact with certain proteins to form highly stable complexes (29, 30, 76) would suggest that an additional apparent fibre component is found in the gastrointestinal tract of chicks fed condensed tannins and that this component, which is probably a tannin-protein complex comprises part of the fibre fraction. As a result the apparent fibre content is elevated.

Feeding tannins leads to low energy conversion, low digestibility of organic material and excretion of high levels of nitorgen (42). Non-specific binding probably accounts for a high inhibitor effect of free tannins on digestive enzymes. Part of the high nitrogen excretion may be endogenous enzyme protein similar to the results of Glick and Joslyn (27). Most of the undigested protein excreted from chicks fed raw hulled faba beans is probably of dietary origin similar to results reported for the feeding of raw soybean (68).

Schingoether (60) has reported compounds of less than 5,000 molecular weight and probably mostly between 1,500 to 800 molecular weight cause possibly 60% of the growth inhibition of raw soybean. These compounds seem to have similar properties to the toxic constituents in faba beans since both seem to prevent the absorption of protein in the intestinal tract. Heated and raw soybeans have similar digestion patterns in the duodenum but net absorption beyond the duodenum is 20% lower for raw soybean fed chicks compared to heated soybean fed chicks. This may be similar to the absorption of proteins in the chick when raw whole faba beans are fed. It is known that tannins can combine with protein reversibly by hydrogen binding or irreversibly by oxidation of the phenols to quinones followed by covalent condensation and polymerization (42). They can therefore form strong bonds with any protein and form relatively insoluble, indigestible complexes.

There are two common tannin phenolics known, condensed tannins and hydrolyzable tannins. The flavanoid type or condensed tannin contains phenolic hydroxyl groups as reactive hydroxyl groups also contain carboxyl reactive groups. The binding of tannins consists largely of hydrogen bond formation between the phenolic hydroxyl group of the tannin and the carbonyl groups of the protein peptide bonds with the bond between the oxygen of the carbonyl group and the tannin supplying the hydrogen molecule (42). Condensed tannin inhibition of trypsin, α-amylase and lipase was determined and it was found to change V_{max} of crystalline trypsin and α-amylase indicating non-competitive reaction kinetics (70). It has been reported that certain amino acids namelu proline, glycine and glutamic acid have specific detoxifying effects on tannins due to selective binding of these amino acids (13). This does not agree with the present data that all amino acid retentions were aggected to a similar degree by faba bean tannins.

The faba beans tested contained only condensed tannins. The condensed tannins and the corresponding low molecular weight components obtained from the acetone-water soluble chromatography were partially characterized. The identity of the descrete compounds of fraction A were not established except that two components were phenolic amino acids, one which was dehydroxyphenyl alanine and the other tyrosine.

The identity of some of the components of the faba bean tannin (fraction B) were investigated. It is known that chemical treatment will convert structural units of condensed tannin molecules into red-purple colored anthocyanidin pigments. In the case of faba bean tannins, four pigments have been isolated and two of these have been identified as being delphinidin (3, 5, 7, 3', 4' Hexahydroxyflavylium chloride) and cyanidin (3, 3', 4', 5, 7-Pentahydroxyflavylium). Purified condensed tannins do not contain any amino acids which would suggest that its structure has not been modified because of an interaction with protein. It has been demonstrated that gallic acid which is a product of the hydrolyzable tannins is not present in the faba bean tannins. The properties of faba bean condensed tannins as obtained in this study are similar in many respects to those reported by Strumeyer and Malin (66).

Cultivar and the length of time the faba bean has been stored have an effect on the amount of measureable tannin material present. Prolonged storage of the bean associated with ripening and darkening of the bean markedly decreases the amount of measureable tannins. Of the cultivars tested in this study at least three had no measureable tannin levels. These varieties were white flowered and light seeded and yielded a non colored water extract. They were similar in properties to those reported by Bond (8) which were shown "in vitro" studies to be more highly digestible than the high tannin dark colored varieties.

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