THE PRODUCTIVE PERFORMANCE OF LAYING HENS FED DIETS CONTAINING FABABEANS INCLUDING STUDIES LEADING TO ISOLATION, PURIFICATION AND IDENTIFICATION OF THE EGG WEIGHT DEPRESSING FACTOR IN FABABEANS

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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

The effect of feeding high levels (40%) of heat treated fababeans, various levels (15 and 30%) of whole and dehulled fababeans which represented different energy levels and methionine supplemented fababeans (25%) to laying hens on productive performance was evaluated. Egg production was not affected ($P>0.05$) by the fababean content of the diet. A consideration of both egg weight and total egg mass results revealed a lower egg size with the fababean diets which was not corrected by heat treatment of fababeans but was significantly ($P<0.01$) improved by methionine supplementation at levels over and above the NRC (1971 and 1977) requirements for the laying hen. The extent of egg weight depression was proportional to the level of whole and dehulled fababeans added to the diet. Feed intake *per se* was not responsible for the observed egg size depression. In general fababean diets were utilized as efficiently as control diets. Body weight gain which was not ($P>0.05$) affected in all trials, tended to increase with increases in the level of methionine in the fababean containing diets. Mortality was normal.

Due to the observed consistency of the egg weight depressing effect of fababeans through the production trials, short term (32 days in length or less) trials were conducted and a standard bioassay test procedure was developed to determine the nature of the component in fababeans that caused

the depression in egg weight. This test procedure consisted of groups of individually caged hens, with one group designated as the control group and the other group(s) as treatment. Control diets were fed to the control group at all times whereas fababean diets were fed to the treatment group(s) during the test periods which were separated by a control period during which the control diet was refed. Eggs were collected and egg weight was recorded daily. The effects of the treatments were evaluated by representing the data graphically and by statistical analysis which involved a comparison between groups of the percent change in egg weight during a test period in relation to a preceeding control period. Energy or protein level in the diet per se was shown not to be a major factor in egg weight response. Addition of 12% untreated or autoclaved (121°C for 10 min) fababean protein concentrate to a laying diet depressed ($P<0.01$) egg weight. Fababean hulls (10% of the diet) or fababean starch (26% of the diet) did not ($P>0.05$) depress egg weight. These results ruled out the possibility of trypsin inhibitors, hemagglutinins or condensed tannins being involved in egg weight depression. An ethanol-water extract of untreated or autoclaved fababean protein concentrate depressed ($P<0.01$) egg weight. The magnitude of the egg weight depression was dependent on the concentration of the causative agent. Fractionation of the ethanol-water extract of autoclaved fababean protein

concentrate by lowering pH using HCl produced fractions which did not ($P>0.05$) depress egg weight. On the contrary, acetone fractionation of the extract produced a fraction (supernatant-H) that depressed ($P<0.05$) egg weight. Supernatant-H was composed of 84.09% of a very soluble fraction (supernatant) and only 11.45% of a relatively insoluble fraction (white precipitate). Both fractions depressed ($P<0.01$) egg weight, although the concentration of the active component was higher in the white precipitate. The white precipitate which contained 80 to 90% total vicine was recrystallized in water to produce crystals which by determination of various physical properties were identified as vicine crystals, containing 96% vicine. Convicine was detected in the residue of crystallization. It was concluded that total vicine (vicine + convicine) is the egg weight depressing factor in fababeans with vicine being the major principle. Chemical analysis of fababean fractions showed that the fababean fractions, hulls and starch that did not ($P>0.05$) depress egg weight contained little or no vicine activity while the potency relative to egg weight depression of the other fractions was proportional to vicine activity.

INTRODUCTION

Fababeans (*Vicia faba*) have been used as human food and animal feeds in a number of countries but primarily in Europe, Middle East and North Africa. Their use in America has been very limited. In Canada, the first commercial production of fababeans occurred in Western Canada in 1972, when about 800 hectares (2,000 acres) were planted and a further expansion was expected in the following years. Average yields exceeding 2,200 kg/ha have been obtained and should increase as producers become more familiar with the crop. The improved varieties of fababeans may well be of considerable potential as protein sources since their protein content is 2½ times higher than that of cereal grains, concurrent with a much higher percentage of lysine. Fababeans have the further advantage of possessing the ability to fix atmospheric nitrogen in the soil, thus benefiting the succeeding crop.

The use of fababeans as a feedstuff, however, is still limited. With regard to their use in poultry feeds, research work has been conducted with growing chickens and to a lesser extent with laying hens. In some laying hen studies egg production has been shown to decrease with high levels of fababeans in the diet. The most consistent effect of adding fababeans to laying diets, however, has been that of egg weight depression.

The objectives of these studies were to evaluate the nutritive value of fababeans for laying hens and in particular to study the alleged egg weight depressing effect of fababeans. In this regard, an attempt was made to isolate, purify and identify the egg weight depressing factor in fababeans.

LITERATURE REVIEW

Types and Varieties of Fababeans

Vicia faba L. is a large seeded annual legume species which can be classified into three types based on seed size and shape (Robinson 1968). *Vicia faba* var. major, has a flat seed sometimes weighing over 0.8 g and is commonly called broad bean or windsor bean. Horse bean (*Vicia faba* var. equina) seed is of medium size, often 0.4 to 0.5 g per seed and has an irregular, oval shape. Tick bean or pigeon bean (*Vicia faba* var. minor) seed is the smallest of the three types (0.2 to 0.3 g) and of a round to oval shape. In Canada and the United States, the common name that has been selected for the species *Vicia faba* is fababeans. These same beans are called field beans in England and silkworm beans in parts of China. Among the new licensed varieties of fababeans are Ackerperle, Diana, Erfordia in Canada; Maris bean and Minor's tick (spring varieties) and Throws M.S. (winter variety) in the United Kingdom.

Nutrient Composition of Fababeans

Improved varieties of fababeans contain about 25 to 35% protein (N x 6.25) on dry matter basis depending on the season, condition of maturity, location, type and variety of the bean (Eden 1968; Clarke 1970; Bhatty 1974; Marquardt *et al.* 1975; and Blair 1977). In Britain, the average protein content was found to be 31.4% for spring beans and 26.5% for winter beans, with lower protein values associated

with large seeded or early maturing varieties. The protein content of Canadian beans appears to be slightly higher than that of British beans (Blair 1977). The bean can be readily separated by mechanical methods into hull (testa) and cotyledon fractions. The cotyledon is about 86 to 89% of the bean while the hull is about 11 to 14% of the bean. The major differences between the hull and the cotyledon are that the former contains 45 to 54% crude fiber and 6 to 7% crude protein, whereas the latter has 0.3% crude fiber and 36% crude protein (Marquardt et al. 1975).

Fababeans are similar to other legumes in that they have a relatively higher level of lysine compared to wheat, but are low in the sulfur containing amino acids, methionine and cystine (Kakade 1974; and Blair 1977). The balance of the other amino acids present in fababeans appears to be good with a few exceptions. Arginine and aspartic acid are higher in fababeans than in wheat, whereas glutamic acid and proline are low (Evans et al. 1972). The sulfur amino acids, glycine and tyrosine are lower in the cotyledon than in the hull portion of the bean, a reverse pattern, however, was observed with arginine and glutamic acid (Marquardt et al. 1975). Kaldy and Kasting (1974), converted the amino acid composition to protein scores to obtain an estimate of protein quality and reported scores varying from 36 to 45 for different varieties of fababeans with an average score of 40 in comparison to an ideal protein with a score of 100. Palmer and Thompson (1975), reported chemical scores and biological

values (B.V.) for four varieties of Vicia faba L. to range from 45 to 52 and 45 to 51, respectively. The protein scored pointed out that methionine is the first limiting amino acid in fababeans. Methionine and cystine values reported for Vicia faba L. range from 0.54 g to 0.95 g per 16 g of N for methionine and 0.78 g to 1.37 g per 16 g of N for cystine (Evans et al. 1972; Frolich et al. 1974; Kaldy and Kasting 1974; and Marquardt et al. 1975). Most of the nitrogen is present as protein and only about 4% as free amino acids, mainly arginine and histidine.

The fat content of fababeans is only 1 to 2% (Carpenter and Johnson 1968; Eden 1968; and Marquardt et al. 1975). More than 97% of the fat is contained in the cotyledon (Blair 1977). Linoleic acid content is reported to be 0.7% compared to 0.4% and 0.9% in soybean meal and barley, respectively (Blair 1977). Fababeans contain 3 to 4% ash and are high in phosphorus and potassium (White 1966; Carpenter and Johnson 1968; Eden 1968; and Marquardt et al. 1975).

The carbohydrates of fababeans were investigated by a number of workers (Prichard et al. 1973; Bhatty 1974; Cerning et al. 1975; and White 1966). The carbohydrate content of fababeans varied from 51 to 66% with 28 to 42% starch. Prichard and associates (1973), reported that the various carbohydrate fractions contained in winter and spring beans were; an available carbohydrate fraction (dextrins, water-soluble and insoluble starches and ethanol

soluble sugars) 46 to 48% and 30 to 42%, respectively; and the unavailable carbohydrate fraction (lignin, cellulose, hemicellulose and water-soluble polysaccharides) 19 to 20% and 22 to 37%, respectively. Only small amounts of glucose containing polymers, soluble in dilute acid, are present in the cotyledon as compared with a value of 3.6% in the hull (Cerning et al. 1975).

Waring and Shannon (1969), determined the classical metabolizable energy (M.E.) values for soybean and two varieties of Vicia faba L., minor's tick and throws M.S. using colostomised laying hens. They reported the M.E. values to be 2.6, 2.5 and 2.4 kcal/g for soybean meal, minor's tick bean and throws M.S. bean meal, respectively. True digestibility coefficients for the crude protein of the beans were 90, 84 and 81%, respectively; the amino acid digestibility coefficients were generally close to those of crude protein. Edwards and Duthie (1970), reported a mean classical M.E. value of 2.4±0.1 kcal/g and a mean nitrogen corrected value of 2.3±0.1 kcal/g by feeding eleven samples of throws M.S. variety of Vicia faba L. to broiler chicks. Comparing the throws M.S. (winter beans) with maris bead tick beans (spring beans), Edwards and Duthie (1970), found the mean classical and nitrogen corrected M.E. values to be 2.4±0.2 and 2.3±0.2 kcal/g for the former and 2.4±0.1 and 2.4±0.1 kcal/g for the latter. They found no relationship between soil types and M.E. values. Carpenter and Johnson

(1968), reported the M.E. value of the spring-sown minor's tick beans to be 2.7 kcal/g and that of the winter-sown throws M.S. beans to be 2.5 kcal/g at 90% dry matter.

Undesirable Constituents of Fababeans

Fababeans have been reported to contain trypsin inhibitors, hemagglutinins, tannins, vicine, convicine and L-dihydroxyphenylalanine (L-DOPA). Some of these antinutritional factors have been implicated in the depressed productive performance of growing chickens fed fababeans while the antinutritional effects of others in poultry is not yet known. Literature relating to each of these undesirable constituents of fababeans is, therefore, cited in an attempt to establish their significance in the nutritive value of fababeans for laying hens.

1. Trypsin inhibitors

A number of workers have reported the presence in fababeans of trypsin inhibitors. Wilson and associates (1972), reported that the pancreas of female broiler chicks was enlarged and feed efficiency reduced when trypsin inhibitor isolate from fababeans was added to a ration containing 75% autoclaved beans, and was enlarged more when raw beans replaced autoclaved beans. Weight gains were depressed by raw beans but not by the isolate. The above workers also presented *in vitro* evidence of the presence of a heat-labile inhibitor of trypsin in both the cotyledon and testa of the field bean. Marquardt and co-workers

(1975), showed that the level of trypsin inhibitor in the hull was at least twofold greater than in the cotyledon. However, trypsin inhibitor in fababeans is much less important than that present in soybean meal, since the level is 5 to 9 fold lower in the fababeans as compared to soybeans. Marquardt et al. (1975), presented evidence which suggested that the trypsin inhibitor in fababeans had a relatively insignificant effect on chick growth and efficiency of feed utilization. The activity of the fababean trypsin inhibitor is destroyed by heating the bean at 110°C for 40 minutes or at 121°C for 15 to 30 minutes (Wilson et al. 1972; and Marquardt et al. 1975).

2. Hemagglutinins

Hemagglutinins (lectins) are substances which have the ability to agglutinate red blood cells. Marquardt et al. (1975), found hemagglutinin levels to be 2,900 to 4,200 rabbit RBC units per gram in four cultivars of raw fababeans, and the levels were reduced to 0 to 300 units after 45 minute autoclaving. The corresponding values for soybeans, wheat and barley were 650, 50 and 5, respectively, and all were reduced to zero by autoclaving for 15 minutes. All the hemagglutinin activity seemed to be concentrated in the cotyledon portion of the bean with no activity in the hull portion (Marquardt et al. 1975).

Hemagglutinins isolated from legumes have been shown to be toxic when injected into animals and to inhibit

growth when added to diets. Pusztai and co-workers (1975), observed that the growth reduction in rats associated with the protein from the phaseolus bean could be explained by the content of hemagglutinins. Some workers have associated the poor growth observed with fababeans to the hemagglutinin content of the beans (Livingstone et al. 1970; Wilson et al. 1972; and Marquardt et al. 1975). More recently studies by Marquardt and associates (1976), however, have demonstrated that hemagglutinins in fababeans do not significantly affect chick growth rates or efficiency of feed utilization. These conclusions were based on the observation that the activity level of the principle growth inhibitor of fababeans following autoclaving at 121°C for 20 minutes was not markedly reduced. Hemagglutinin activity levels in contrast, were greatly reduced. It was also observed that the major portion of the growth inhibitory activity was found in the testa portion of the bean, a fraction that contained no hemagglutinin activity.

Although the role of hemagglutinins in favism, a disease shown to result from eating fababeans, is not known, hemagglutinins are suspected as being one of the causative agents. Raw uncooked beans appear to be most dangerous while cooked fresh beans are less likely to produce attacks and cooked stored beans are least hemolytic (Motulsky 1972). In several experiments decreased glutathione (GSH) levels were observed in glucose-6-phosphate dehydrogenase (G-6-PD)

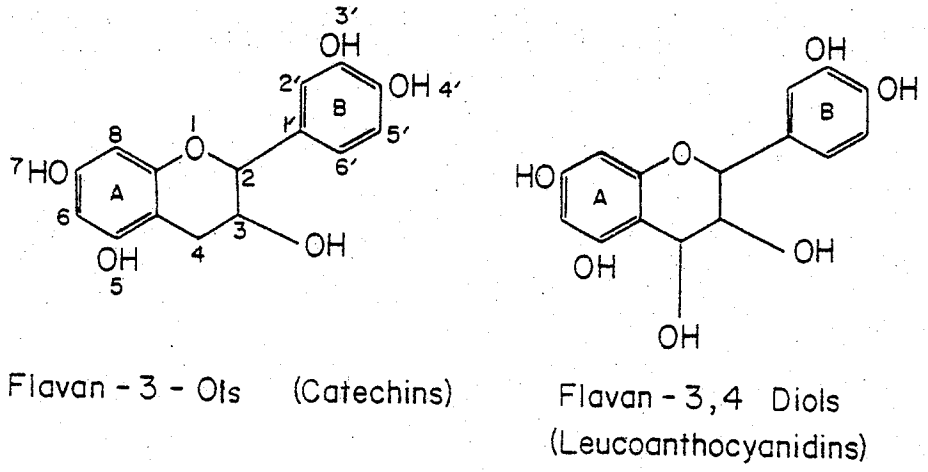
deficient red blood cells in the presence of broad bean juice, crude aqueous extract of fababeans, saline extracts of faba pollen and pistils and lipid soluble materials from fababeans (Mager et al. 1969). Sharon et al. (1972), stated that Vicia faba contains a non-specific lectin (cell-agglutinating protein) that has certain sugar specificities and the ability to agglutinate blood groups of all types. This property might be associated with the binding of a sugar molecule, specifically D-mannose and D-glucosamine on the erythrocyte wall which may influence the further activity of the cell in G-6-PD deficient erythrocytes. The nature and properties of lectins are so varied and complex that little is known about their actual function in plants and its relationship to other aspects of biochemistry (Sharon et al. 1972). The possibility of a lectin being one of the causative agents in favism is not well documented.

3. Tannins

Tannins have been classified into two groups, the hydrolyzable and condensed tannins. They have in common protein-binding and leather-forming activities, but they usually differ considerably in botanical distribution, physiological properties and breakdown products. Tannic acid is typical of the hydrolyzable tannins. It is readily hydrolysed enzymatically or spontaneously to glucose and gallic acid. The condensed tannins (flavolans) are polymeric flavanoids composed predominately of leucoanthocyanidin

units linked carbon-to-carbon from the 4 position of one unit to the 6 or 8 position of the next. Typical condensed tannin precursors are shown in Fig. 1. These do not break down readily under physiological conditions; when treated drastically, they usually produce either less soluble polymeric phlobaphenes or flavonoid monomers, particularly catechin and anthocyanidin as in Fig. 1 (Singleton and Kratzer 1973). These phenolic compounds combine with proteins reversibly by hydrogen bonding and irreversibly by oxidation followed by covalent condensation (Loomis and Battaide 1973).

Condensed tannins are present in some plant materials at very high levels (Singleton and Kratzer 1965). They have been reported to be of significance in some common feedstuffs such as sorghum grains (Chang and Fuller 1964); and fababeans (Marquardt et al. 1977). Chang and Fuller (1964), found that feeding sorghum grains with high tannin content produced a reduction in chick growth. Fuller et al. (1966), showed that milo sorghum containing 1.6% or more tannins depressed chick growth when fed at 50% of the diet. Hughes (1973), reported that a dietary level of 0.05% tannins from sawdust resulted in mottled yellow yolk in chicken eggs while 0.5% tannins gave olive green yolks. Rostagno et al. (1973), found that feeding high tannin, bird-resistant sorghum gave poorer weight gain and feed conversion than bird-susceptible, low tannin sorghum. The addition of polyvinylpyrolidone (PVP), a substance that binds tannins, to a diet that contained bird-resistant sorghum improved chick growth



Flavan - 3 - Ols (Catechins)

Flavan - 3,4 Diols (Leucoanthocyanidins)

Fig. 1. Typical condensed tannin precursors.

and feed efficiency. The addition of 0.15% methionine to both bird-susceptible sorghum and bird-resistant sorghum also resulted in a significant improvement in chick performance. The response was greater with the bird-resistant compared with the bird-susceptible sorghum grain diets, although feed efficiency of the bird-resistant sorghum remained poorer. Rostagno et al. (1973), reported the apparent amino acid digestibilities of low, intermediate and high tannin sorghum varieties to be 74, 41 and 22%. Eggum and Christensen (1975), feeding increasing amounts of tannins to rats with soybean meal as the protein source demonstrated that tannins exerted a severe negative effect on protein digestibility and that the availability of methionine was more severely affected than the availability of the total nitrogen. Astringency resulting from feeding high tannin sorghum grains is thought to result from binding of mucosal proteins in the mouth by tannins, and addition of protein counteracts this effect. Strumeyer and Malin (1975), isolated tannins from sorghum on LH-20 Sephadex. The tannins were shown to consist of a series of polymeric polyphenols which upon acid hydrolysis generated cyanidin exclusively.

Marquardt et al. (1975), found that more than one-half of the total chick growth inhibiting potential of the whole fababean was associated with the hull. Bond (1976), reported that white-flowered tannin-free varieties of

Vicia faba showed increased in vitro digestibility compared to colored-flowered varieties of similar seed size. The difference was shown to be largely due to the digestibility of the organic matter of the testa in tannin-free varieties of 56.4% compared to 17.2% in the tannin-containing varieties. Marquardt et al. (1977), have isolated the chick growth depressing factor from the hull portion of the fababean using Sephadex LH-20 column, and have identified the active component as a condensed tannin. This thermolabile factor, which is soluble in water and ethanol, markedly depressed nutrient intake and reduced nutrient retention, particularly protein. The net effects were depressed rate of growth of chicks. These workers have also reported that the present licensed Canadian varieties of Vicia faba minor, when grown under similar environmental conditions, have similar condensed tannin levels. An analysis of more than 20 fababean cultivars of widely differing genetic origins and from several different countries indicated that condensed tannin levels within these cultivars did not vary greatly. The above workers, however, found three cultivars of "triple white" broad beans (Vicia faba major) to be free from condensed tannins. These cultivars also had a low level of polyphenolic compounds and did not contain the compounds which cause the bean to darken on storage.

4. Vicine, Convicine and L-DOPA

Vicine and convicine were first isolated by Ritthausen (1881), in Vicia sativa. These compounds were subsequently found in other species of Vicia including Vicia faba (Mager et al. 1969). The pyrimidine nucleoside structure was assigned to vicine and convicine by Johnson (1914). The correct formulation of vicine as 2, 6-diamino-4, 5-dihydroxy pyrimidine, 5-(β-D-glucopyranose) and convicine as 2, 4, 5-trihydroxy, 6-aminopyrimidine, 5-(β-D-glucopyranose) was established by Bendich and Clements (1953).

In search of the favic causative agent in fababeans, Mager et al. (1969), was aided by the fact that the agent had a capacity for oxidizing glutathione (GSH) in glucose-6-phosphate dehydrogenase (G-6-PD) deficient red blood cells but not in normal cells. Extracts from fababeans conformed to this criterion and some were sparingly soluble in water and exhibited in neutral solutions a loss of GSH oxidizing activity (Lin 1963; and Mager et al. 1969). These fractions had a tendency to undergo spontaneous oxidation in air (Mager et al. 1969). The properties of the active fractions appeared similar to some pyrimidine derivatives known to occur in fababeans in the form of aglycones of β-glycosides termed vicine and convicine, the former being present in greater amounts than the latter (Mager et al. 1969; Higazi and Read 1974). The aglycones, divicine and isouramil can be obtained from the respective glycosides vicine and

convicine by mild acid hydrolysis or by enzymatic splitting with β-glycosidase as shown in Fig. 2, (Mager et al. 1969).

The aglycones are highly unstable in the presence of oxygen and are almost instantaneously destroyed by boiling. The rate of their breakdown is most rapid at alkaline pH and falls with decreasing pH values (Mager et al. 1969). At room temperature the half-life is in the order of 30 to 40 minutes. The breakdown of the pyrimidine aglycones is accelerated by traces of copper (Cu^{++}) and other heavy metals (Mager et al. 1969). All the characteristic properties of the aglycones are abolished by substitution of the hydroxyl group at C-5, such as that represented by the glycosidic linkage present in vicine and convicine (Mager et al. 1969). These glycosides show none of the reducing properties of their aglycones, are heat stable and their ultraviolet spectra are different from those of their aglycones (Mager et al. 1969).

Both divicine and isouramil reduce alkaline solutions of 2, 6-dichlorophenolidophenol, ammonium phosphomolybdate or phosphotungstate and elicit an intense blue color reaction with ammoniacal ferric chloride solution (Bendich and Clements 1953; and Mager et al. 1969). Some of the distinctive features of divicine and isouramil such as their powerful reducing properties, spectral characteristics and molecular instability are similar to those of ascorbic acid (Mager et al. 1969).

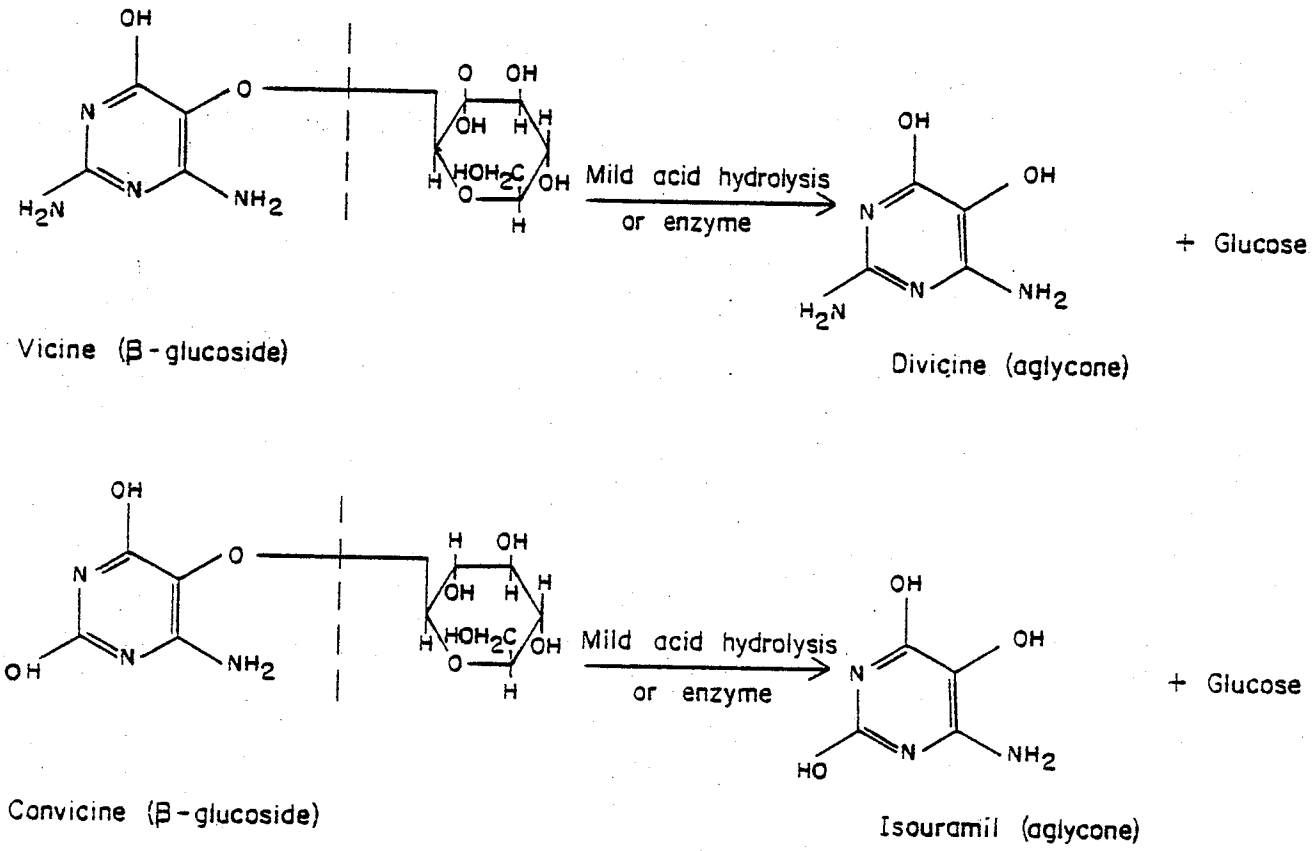


Fig. 2. Production of divicine and isouramil from vicine and convicine, respectively.

Incubation of human red blood cells with the aglycones, divicine and isouramil resulted in a rapid fall in their glutathione (GSH) level. Addition of glucose prevented the injurious action on normal erythrocytes but not glucose-6-phosphate dehydrogenase (G-6-PD) deficient ones (Mager et al. 1969). The powerful capacity for oxidizing GSH exhibited by the pyrimidine aglycones in vitro is consistent with a possible causative role of these substances in precipitating the favic crisis (Lin and Ling 1962; and Mager et al. 1969). The free aglycone may arise from the parent glycosides either in the bean or in the digestive tract through the hydrolytic action of β-glucosidase (Mager et al. 1969). The conditions present for enzymatic release of aglycones from the glycosides, as well as the particular instability of these aglycones might account for the puzzling irregularity which characterizes the occurrence of favism in susceptible individuals, irrespective of the degree and frequency of their exposure to the noxious agent (Mager et al. 1969).

L-dihydroxyphenylalanine (L-DOPA), has recently gained a prominent place in the treatment of Parkinson's disease (Beutler 1970 and Longo et al. 1973). Faba-beans are the major source of large amounts of free L-DOPA, and are the source from which L-DOPA is produced commercially (Beutler 1970). They may contain 0.25% of L-DOPA by weight (Razin et al. 1968). L-DOPA and tyrosine levels are highest

in fababeans during the fruiting period (0.56% and 0.044%) and are lowest in the seed (0.075% and 0.01%) (Longo et al. 1973). Mager et al. (1969), stated that 3, 4-dihydroxy phenylalanine (L-DOPA), may be one of the active factors responsible for the ability of fababeans to induce hemolysis in G-6-PD deficient individuals. Kosower and Kosower (1967), found that concentrations of L-DOPA as low as 0.75 μ mole/ml of a glucose incubation mixture could produce significant losses of GSH in G-6-PD deficient red cells. Razin et al. (1968), failed to find an effect even when 10 μ moles of L-DOPA were added to each ml of the reaction mixture. On the other hand a combination of 1 mM L-DOPA and 0.2 mM isouramil resulted in an 80% destruction of GSH (Mager et al. 1969). Similar results were obtained with sodium ascorbate and isouramil (Mager et al. 1969). Mager et al. (1969), suggested that L-DOPA and ascorbic acid might act synergistically with substances such as vicine and convicine in the pathogenesis of favism. Beutler (1970), noted that alkali-treated L-DOPA was much more effective as an oxidant of GSH than native L-DOPA.

Dopaquinone has also been suggested as being the active hemolytic principle in fababeans (Beutler 1970). Susceptibility to the hemolytic effect of fababeans may depend upon the rate of its production by oxidation of tyrosine through the action of tyrosinase as shown in Fig. 3 (Beutler 1970). L-DOPA alone had little or no effect on

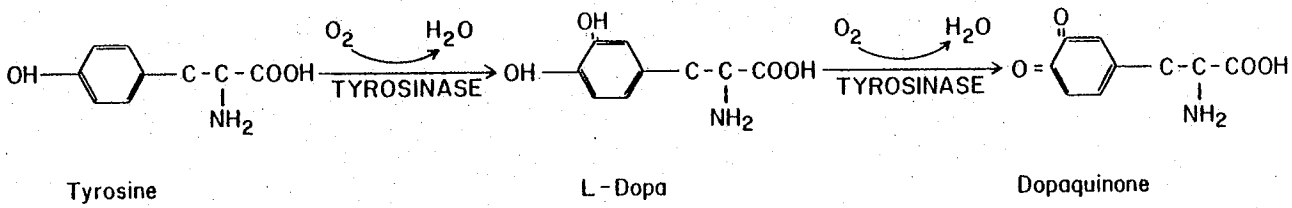


Fig. 3. Oxidation of tyrosine to dopaquinone.

red cell GSH levels. The addition of tyrosinase resulted in the conversion of L-DOPA into a substance, presumably dopaquinone, which had the capacity to partially deplete G-6-PD deficient erythrocytes of GSH (Beutler 1970). Beutler (1970), concluded that if dopaquinone is the active principle in fababean induced hemolysis, it may then be possible to explain the fact that some G-6-PD deficient individuals are susceptible to favism, while others are not. He suggested that a polymorphism of the enzyme system required for the conversion of L-DOPA to dopaquinone, the tyrosinase system, could easily explain such individual differences. Furthermore, the differences in response to fababeans could also be due to differences in the rate of metabolism and elimination of dopaquinone formed from L-DOPA of the beans, or in the rate of decarboxylation of L-DOPA.

Favism

The term "favism" was coined in 1814 by the Italian physician L. Montano to designate an acute hemolytic anemia following ingestion of broad beans (fababeans) or inhalation of pollen of the Vicia faba plant (Sansone et al. 1958; and Mager et al. 1969). Prominent symptoms of hemolytic anemia are weakness or fatigue, vomiting, jaundice, and destruction of the red blood cells (Kavoura 1954; and Besley 1973). The course of the disease is usually self-limited,

the acute stage lasting 24 to 48 hours, and then followed by prompt spontaneous recovery. The hemolysis is also of variable intensity and gravity, the more severe cases being attended by hemoglobinuria and jaundice (Du 1952; and Mager et al. 1969). Fresh raw beans appear to be most dangerous, dry or cooked fresh beans are least hemolytic (Luisada 1941; Mager et al. 1969; and Motulsky 1972). Epidemiological studies in Italy revealed the seasonal incidence of the disease, characterized by two peaks; in April to May, when the plant blossoms, and in July to August, when the fresh ripe beans appear on the market (Luisada 1941; and Mager et al. 1969). The therapy of treatment is usually blood transfusion in severe cases (Crosby 1956; Stamatoyannopoulos et al. 1966; and Mager et al. 1969).

There appears to be a definite geographical distribution of favism. It is more prevalent in the Middle East areas and the Mediterranean areas of Sardinia, Sicily, Southern Italy, Greece, Turkey, Levant, Israel, Spain, and Balearic Islands. The highest incidence has been observed in Sardinia. In Europe, outside the Mediterranean coast, America and other parts of the world, cases of the disease are rare (Crosby 1956; and Mager et al. 1969).

Red blood cell destruction due to fababeans has been attributed to enzymatic abnormalities in red cell metabolism affecting the pentose-phosphate pathway and glutathione metabolism (Motulsky 1972). The most common abnormality is glucose-6-phosphate dehydrogenase (G-6-PD)

deficiency, a sex ($\bar{x}$) linked genetic defect (Mager et al. 1969; and Motulsky 1972). The susceptible red blood cells exhibit a relatively low content of reduced glutathione (GSH) and enhanced rate of GSH destruction on incubation with oxidant compounds such as primaquine, acetylphenylhydrazine, divicine and isouramil (Mager et al. 1969). This is due to the fact that G-6-PD deficiency results in an inadequate supply of NADPH. NADPH is normally produced when glucose-6-phosphate is diverted to the pentose shunt pathway in the presence of NADP and G-6-PD when the body has an excess supply of energy according to the following scheme:

Glucose-6-phosphate + NADP dehydrogenase> 6-phosphogluconate + NADPH + H^+

6-phosphogluconate + NADP dehydrogenase> Ribose-5-phosphate + NADPH + H^+

GS-SG + NADPH + H^+ reductase> 2 GSH + NADP.

The NADPH is used to convert oxidized glutathione (GS-SG) to the reduced state (GSH) (Liener 1969).

The G-6-PD deficient red blood cell is able under normal conditions to maintain an adequate cellular level of GSH despite the reduced G-6-PD activity and the limited capacity of GS-SG reductase to use NADH as an alternative hydrogen donor (Brewer et al. 1961; and Mager et al. 1969). This precarious metabolic equilibrium, however, breaks down under the stress conditions imposed by an oxidant compound resulting in irreversible oxidation of GSH and catabolism of GS-SG, ultimately leading to the destruction of the

enzyme-deficient red blood cell (Beutler 1957; and Mager *et al*. 1969). A major manifestation of the oxidant action of the drugs both *in vivo* and *in vitro* is the formation of methemoglobin and the appearance of Heinz bodies, which represent a denatured product of hemoglobin destruction resulting from oxidation of its SH-groups (Allen and Jandl 1961; and Mager *et al*. 1969).

GS-SG has been shown to inhibit the activity of a number of enzymes including G-6-PD, inorganic pyrophosphatase, triosphosphate dehydrogenase, hexokinase and ATPase (Mager *et al*. 1969). Incubation of red blood cells in the presence of acetylphenylhydrazine (APH), divicine, isouramil and primaquine, resulted in pronounced inhibition of glycolysis (Loehr and Waller 1961) and a progressive decrease in the ATP level of the cells (Mager *et al*. 1969). Addition of glucose alleviated the deleterious effects of drugs in normal but not in G-6-PD deficient cells.

It is estimated that G-6-PD deficiency affects over 100 million people all over the world (Mager *et al*. 1969; and Motulsky 1972), but a definite ethnic distribution is noted (Mager *et al*. 1969). The occurrence of favism does not parallel the frequency of the G-6-PD deficiency trait in different populations (Mager *et al*. 1969).

As the G-6-PD deficiency is sex-linked, the enzyme deficiency is fully expressed in the hemizygous ($\overline{x}Y$) male because the mutant gene ($\overline{x}$) is not counteracted by the

normal gene (x). Expression in the female is uncommon as the homozygous mutant genotype ($\bar{x}$ $\bar{x}$) is necessary for full expression (Mager et al. 1969). In the majority of affected females with only one mutant gene ($\bar{x}$ x), the G-6-PD deficiency is of partial or intermediate nature. The degree of susceptibility of these females is dependent upon the proportion of G-6-PD deficient cells which may be from 1 to 90% in a few patients although the usual percentage is 50% (Mager et al. 1969; and Motulsky 1972).

Glucose-6-phosphate dehydrogenase (G-6-PD) has been shown to consist of two identical chains A and B consisting of 420 amino acids with the only difference being the replacement of asparagine by aspartic acid in the A variant (Boyer et al. 1961; Yoshida et al. 1967; and Motulsky 1972). Several variants are possible. Kirkman et al. (1964) and Mager et al. (1969), reported 6 variants, while Motulsky (1972), reported from 80 to 100. Many variants, however, have only mild or no reduction of enzymatic activity, therefore, are not sensitive to fababeans or drugs (Motulsky 1972). Others exhibit from a severe loss to a mild loss of the enzyme activity. The severe cases are without exposure to a noxious agent, while the less severe cases are only sensitive to a deleterious agent (Motulsky 1972).

Most studies have been conducted on the Mediterranean (Caucasian) type B deficiency and the African (Negro) type A deficiency (Mager et al. 1969; and Motulsky 1972).

Whereas the Mediterranean type produces severe cases of favism, the African type produces milder cases or no favism at all (Mager et al. 1969; and Motulsky 1972). The reason for this phenomenon is due to the fact that the African type of deficiency affects mainly the older cells while the Mediterranean type of deficiency affects all the cells (Motulsky 1972). The genetic mutation underlying the African type (A variant) G-6-PD deficiency entails a structural alteration of the enzyme molecule which does not affect its initial catalytic capacity, but enhances its rate of inactivation in the course of cell aging (Mager et al. 1969). In this type of deficiency, young cells (0 to 50 days), have sufficient enzyme activity for normal function and are not affected by offending agents (Motulsky 1972). In the Mediterranean type of deficiency, all cells are deficient in the enzyme and the offending agent destroys a much larger red blood cell population (Motulsky 1972). However, the degree of enzyme deficiency bears no resemblance to the degree of favism (Mager et al. 1969). Stamatoyannopoulos (1966), has postulated that a second autosomal genetic factor is involved. His theory is based on the fact that an overt episode may occur after many years of exposure to Vicia faba, and subsequent re-exposure does not necessarily lead again to favism. Also favism appears to be confined to certain families and regions. Furthermore, the occurrence of a crisis is not dosage

dependent, and it can follow even a trivial exposure. Although this second genetic factor, as G-6-PD deficiency, does not necessarily lead to favism, its influence on the susceptibility of the red blood cells to hemolysis or its modifying effect on the absorption, detoxification or excretion of the causative agent of favism should be considered (Stamatoyannopoulos 1966).

Fababeans in Poultry Rations

1. Rations for growing chickens

The nutritive value of fababeans for chicks has been shown to improve when the beans are dehulled, heat treated and/or supplemented with methionine.

Bletner et al. (1963), reported satisfactory results with chicks fed rations containing 33 or 60% fababeans. They, however, observed poor feathering, relatively high incidence of perosis and lack of yellow pigment in the skin, beak and shanks of birds fed the diets containing 60% fababeans. Blair and Bolton (1968), noted no adverse effects on liveweight gain or feed conversion during the period 4 to 9 weeks when broiler chicks were fed rations containing up to 30% fababeans. On the other hand, Blair et al. (1970), after feeding broiler chicks diets containing 0, 15, 30 and 40% fababeans for 0 to 4 weeks and 0 to 8 weeks, found significantly poorer feed efficiencies and weight gains as the level of fababeans in the diet increased. Wilson and

McNab (1972), fed broiler chicks diets containing 75% raw and autoclaved fababeans in one trial and supplemented these diets with methionine in another trial. They reported that autoclaving was only beneficial when the sulfur amino acids were limiting, and concluded that the beneficial effect of autoclaving was due to increased amino acid availabilities. Grey et al. (1972), fed pelleted rations containing 45% fababeans and reported that birds fed the fababean ration were heavier than the control birds. Edwards and Duthie (1973), demonstrated that autoclaving or infrared radiation of fababeans improved the metabolizable energy values of fababeans when fed to broiler chicks by 12% and 10%, respectively. The same workers also reported that dehulling increased the metabolizable energy of fababeans from 2,280 to 3,033 kcal/kg, a 33% increase. This increase was much higher than expected since the testa is about 13% of the whole bean. It appeared that the hull contained a factor that decreased nutrient utilization in the cotyledon portion of the bean.

Marquardt and Campbell (1973), found the most pronounced effect of heat treatment with diets of higher (85%) fababean content compared to diets of lower (57 or 28%) fababean content. Marquardt and Campbell (1974), determined the productive performance of chicks fed diets (21% protein) containing 87% raw or autoclaved fababeans. They found that methionine supplementation of autoclaved as compared to raw fababean diets improved weight gain by 10%, feed:gain ratio

by 10% and decreased pancreas size by 17%. Liver size was not affected. Optimal productive performance was obtained for birds consuming diets containing 0.21% added methionine. The total dietary methionine and sulfur amino acid levels under these conditions were 0.43% and 0.68%, respectively. In another study, Marquardt and Campbell (1975), reported that chicks fed a ration containing 90% fababeans grew significantly better when 0.24% methionine was added to the diet. This raised the level of methionine in the diet to 0.46% and total sulfur amino acid level to 0.78%. Marquardt et al. (1974), also conducted experiments to evaluate the effects of various autoclaving times (0, 15 or 30 min. at 121°C) on fababean utilization by chicks. All diets which were supplemented with methionine contained 90% fababeans. Autoclaved as compared to raw fababeans significantly improved weight gains, feed:gain ratios; and reduced feed intake and pancreas size. The 30-minute autoclaving time gave greater improvements than the 15-minute autoclaving time. Kadirvel and Clandinin (1974), reported that feeding up to 20% fababeans to broiler chicks from 0 to 4 weeks had no deleterious effect on growth rate and pancreas weight. There was, however, a nonsignificant decrease in weight gain and increase in pancreas weight when 35% raw fababeans were added to the diet. Raw and autoclaved fababeans gave poorer chick weight gains than soybean meal or rapeseed meal, but methionine supplementation of fababean diets improved the growth response of chicks to that of

soybean meal (Sharby and Bell 1974).

The effects of various processing methods on the productive performance of broiler chicks fed diets containing fababeans were variable (Marquardt et al. 1976). Microwave treatment was the least effective and autoclaving or extruding were the most effective of the various processing methods studied. The corresponding increases in weight gain and the improvements in feed:gain ratios for chicks fed raw as compared with processed beans were: microwave treatment for 20 minutes, -1% and -4%; microwave treatment for 30 minutes, 6% and 1%; steam pelleting, 6% and 8%; extruding at 152°C, 16% and 18%; and autoclaving at 121°C for 20 minutes, 17% and 10%, respectively. Studies with individual fababean fractions (starch isolate, protein concentrate and hulls), revealed that processing improved the utilization of fababeans mainly through its effect on components associated with the hull and protein portions of the bean. Processing of the starch fraction did not improve its utilization by chicks (Marquardt et al. 1976). Although the protein and hull fractions appeared to contain a growth depressing factor, this factor was not associated with a trypsin inhibitor, hemagglutinin or vicine (Marquardt et al. 1976). The rather short autoclaving time (15 to 20 min.) required to denature trypsin inhibitor and inactivate hemagglutinin as compared with the much longer period (30 to 40 min.) required to achieve optimal performance suggested that some

other component associated mainly with the hull was responsible for depressed utilization of fababeans by chicks (Marquardt et al. 1976). Vicine was not destroyed by either autoclaving at 121°C for 40 minutes or by extruding at 152°C while the performance of chicks was restored to normal (Marquardt et al. 1976).

A chick growth depressing factor which is soluble in acetone and water was extracted from fababean hulls by Marquardt and associates (1976). This factor which was isolated using Sephadex LH-20 column was identified as a condensed tannin having chemical characteristics similar to tannins present in sorghum grains (Strumeyer and Malin 1975; and Ward et al. 1977). Chicks fed a diet containing 3.9% of the purified condensed tannin as compared to those fed a control diet had markedly reduced feed intake (69%), negative weight gains and feed:gain ratios; and reduced dry matter (22%), amino acid (25%) and crude fiber (175%) retention. Fat retention, in contrast, was increased (27%) (Marquardt et al. 1977; and Ward et al. 1977).

2. Laying hen rations

Some studies on the use of fababeans in laying hen rations have been reported, although the data is limited.

Carpenter and Johnson (1968), reported M.E. values of 2.68 and 2.52 kcal/g for minor's tick bean and throws M.S. bean, respectively, whereas Waring and Shannon (1969), obtained 2.47 and 2.39 kcal/g for the same varieties of

fababeans using colostomized fowl. True digestibility coefficients of the two varieties of fababeans were 84% and 81% (Waring and Shannon 1969). Lanza and associates (1971), fed rations containing up to 30% fababeans and supplemented with up to 0.12% methionine, to pullets from 26 weeks of age. They reported that weight gains were reduced by 32%, egg production was reduced by 12% and egg weight was reduced by 8%. Vogt (1972), observed that while 10% fababeans in laying rations supplemented with methionine had no adverse effects, the use of 20% fababeans in rations for laying hens in floor pens resulted in decreased feed conversion efficiency and smaller eggs. Caged layers fed 25% fababeans also showed poorer performance than hens fed a control ration. Davidson (1973), found that egg production was depressed with 15% fababeans and was improved to about that obtained with a control ration when supplemented with 0.1% methionine. Whether or not methionine was added, the inclusion of fababeans at more than 15% resulted in lowered egg production. Egg weight was depressed when fababeans were included in the ration and the depression was only partly corrected by methionine supplementation. Pelleting the feed improved feed intake and egg production with the bean rations. This worker concluded that no more than 15% fababeans should be used in laying hen rations until more is known about the factors that depressed performance when higher levels were fed. Marquardt and coworkers (1974),

reported reduced egg production and egg weight with a fababean ration in a practical test involving 10,500 layers. In this trial hens were fed a control ration or a fababean ration in which 25% fababeans (plus 0.15% added methionine) replaced 15% barley and 10% soybean meal in the control ration. Wilson and Teague (1974), found that 20% fababeans in laying rations had no effect on egg production or feed intake but caused a significant decrease in egg weight. In a series of two experiments Robblee et al. (1977), fed pullets rations containing 0, 5, 10, 15, 20 and 30% fababeans for 336 days. The results obtained indicated that levels up to and including 20% fababeans in rations supplemented with methionine had no adverse effect on mortality, rate of egg production, efficiency of feed conversion or body weight. A level of 30% fababeans resulted in increased mortality, and decreased egg production and feed conversion in one experiment but had no effect in the other. Egg weight was decreased as the level of fababeans in the rations increased. There was an increase in interior quality of the eggs as measured by Haugh units as the level of fababeans incorporated into the diets increased. In contrast, specific gravity of the eggs was not influenced by diet content of fababeans.

It can be concluded from the above discussion that relatively high levels of fababeans can be fed to growing birds with no decrease in performance as long as the beans are heat treated and the diets are adequately formulated

with regard to methionine and energy contents as well as other nutrients. The information, however, is not that clear concerning the use of high levels (25 to 40%) of fababeans in laying hen diets. This is particularly true with regard to the influence of fababeans on egg weight where a depressing effect is apparent even at relatively low levels of dietary fababeans.

In laying hen feeding the disadvantages of fababeans are: relatively low metabolizable energy (M.E.) and methionine contents, and the presence of several antinutritional substances. The egg weight depression among laying hens fed fababeans and the apparent decrease in productive performance of laying hens fed high levels of fababeans may be associated with one or more of these factors.

In an attempt to assess the significance of dietary energy level on the productive performance of laying hens, Brown (1964), fed diets containing 1,936, 2,200, 2,310 and 2,640 kcal M.E./kg and found significant improvements in feed conversion efficiency when the energy content of the diet increased. The diet lowest in energy content led to a considerable reduction in total energy intake of the pullets which was accompanied by a fall in production. In another study where pullets were fed diets containing 2,574, 2,728, 2,794 and 2,948 kcal M.E./kg, the results showed that energy level had no significant effect on egg production or egg weight. These results would indicate that laying hens

are able to adjust their energy and other nutrient intake as long as the dietary energy level is not less than 2,600 kcal M.E./kg. Practical wheat-soybean meal diets contain approximately 2,700 kcal M.E./kg. Fababeans are reported to contain an average of 2,400 kcal M.E./kg (Waring and Shannon 1969, and Edwards and Duthie 1970), which could lower the energy content of such diets if added at high levels and may be the cause of the observed decreased productive performance, particularly egg weight, of laying hens fed such diets.

A number of workers have conducted experiments in order to establish the methionine requirement of the laying hen. Combs (1964), indicated the methionine requirement to be 295 mg per hen per day, while Bray (1965), estimated it to be 233 mg per hen per day. Harms and Damron (1969), concluded that the methionine requirement was between 250 to 280 mg per hen per day, provided that the diet contained a total of 530 mg of sulfur amino acids. Carlson and Guenthner (1969), found no response when diets containing 16% protein were supplemented with methionine, only when the diets contained 14% protein was the need for methionine supplementation for maximum egg production clearly demonstrated. Calculations for daily nutrient intake showed that 17 g of protein per hen was required without methionine supplementation, whereas 15 g of methionine-supplemented protein was adequate for maximum egg production, egg weight and feed efficiency. The above workers, also indicated that

the methionine requirement was in excess of 300 mg per day during the first four months of production, but between 289 and 328 mg per day during the later stages of the laying cycle. Fisher and Morris (1970), on the other hand, estimated the methionine requirement to be 275 mg per hen per day for maximum egg yield of pullets during the early stages of lay. Gleaves and Dewan (1970), concluded that methionine exerts a greater influence upon production characteristics of laying hens than upon feed consumption per se. The National Research Council (1971), recommends a level of 0.28% methionine and 0.25% cystine in a ration containing 2,850 kcal M.E./kg.

The literature on the use of fababeans in laying hen diets indicated that methionine supplementation of laying diets containing fababeans is a prerequisite for optimal laying hen performance. This could be the result of the relatively low (0.22%) methionine content in fababeans (Waring and Shannon 1969; and Marquardt et al. 1975), coupled with the fact that methionine is the first limiting amino acid in most practical rations fed to laying hens (Jensen et al. 1974). The practical rations referred to by Jensen and the rations mostly used in the establishment of methionine requirement of laying hens are based on soybean meal which contains 0.65% methionine (Scott et al. 1976). It would appear, therefore, that increasing the level of fababeans in diets for laying hens would make such diets

even more deficient in methionine. The methionine requirement, however, has not been established using diets containing a relatively high level of fababeans.

There is no information available on the specific role of the antinutritional factors in fababeans on the productive performance of laying hens. Trypsin inhibitors are generally known to reduce amino acid availability due to their ability to inhibit proteolytic activity. Tannins also reduce amino acid availability by binding protein and reducing protein retention. The role of vicine and related compounds in affecting the nutritive value of fababeans for poultry is not documented.

MATERIALS AND METHODS

INTRODUCTION

A series of experiments were conducted in an attempt to gain additional knowledge regarding the influence of fababeans on the productive performance of laying hens. Initial experiments were designed to evaluate the effects of feeding relatively high dietary levels of fababeans processed in a variety of forms and to study the effects of supplemental methionine. Subsequent experiments were designed with the aim of obtaining information pertaining to the most consistent antinutritional effect of feeding fababeans to laying hens, that of egg weight depression. The ultimate aim of this last series of experiments was the isolation, purification and identification of the factor causing egg weight depression in fababeans.

Fractionation of fababeans either by dehulling, air classification or extraction with various solvents was employed to prepare materials used in several of the experiments.

PREPARATION OF FABABEAN FRACTIONS

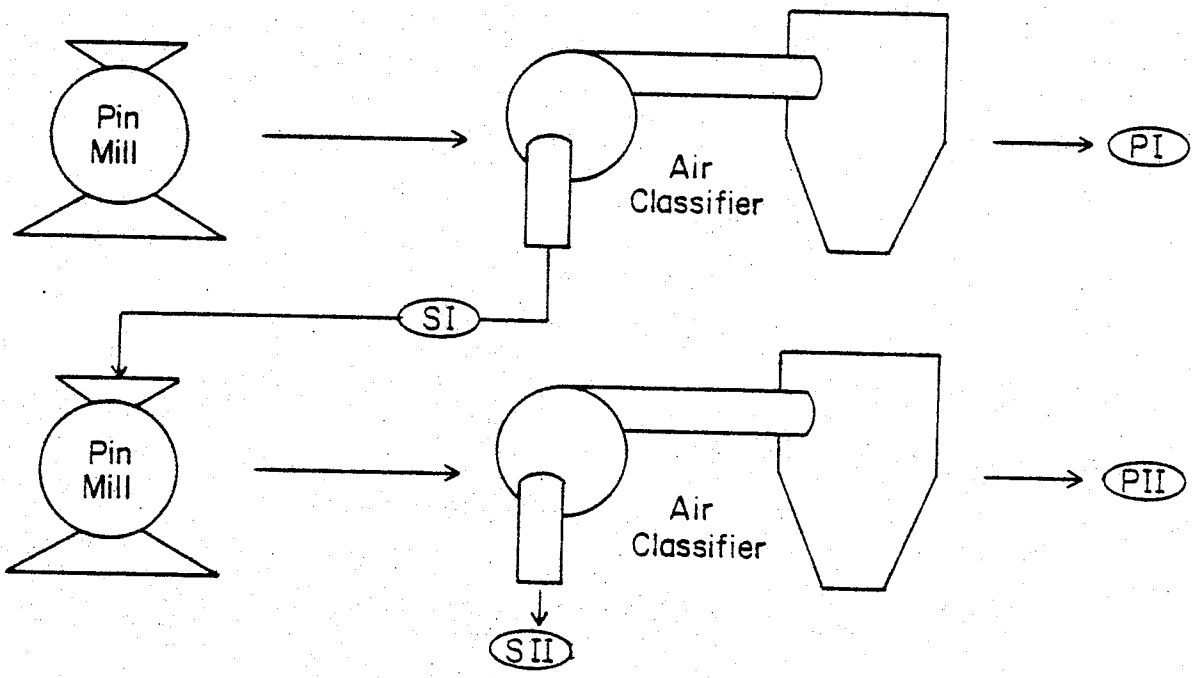
In those experiments where fababean hulls, starch or protein concentrate were used the preparation of the various fractions was essentially as described by Craig (1974) and Vose *et al.* (1976). Fababeans (*Vicia faba* L. var Diana) were cleaned and equilibriated to a moisture

content of 8% prior to processing. The process involved cracking of the hulls with a plate-type grinder and separating of hulls from the cotyledons by air aspiration. The dehulled fababeans (cotyledons) were ground in a pin mill and the protein (less dense fraction) and starch (dense fraction) were separated in an air classifier. The process of air classification of the dehulled beans is illustrated in Fig. 4.

A typical fractionation started with 100 kg of bean flour at 31.9% protein. The first pass through the air classifier yielded 30% of a protein rich fraction at 69% protein and 70% of starch at 16.5% protein. The starch fraction was re-ground in the pin mill and was passed through the air classifier a second time. This process yielded an additional 18% of protein concentrate with a protein content of 49.6%. The double pass system using the pin mill and air classifier yielded 48% of protein concentrate with a protein level of 59 to 60% and 52% of starch with 4 to 6% protein.

ROUTINE CHEMICAL AND STATISTICAL ANALYSES

Proximate analysis of the diets and fababean fractions was carried out by methods according to the Association of Official Agricultural Chemists (A.O.A.C.) (1970). Amino acid analyses were performed by the method of Moore and Stein (1963) with a Beckman model 119C automatic



FLOUR	100 Kg	(31.9 % protein)
SI	70 Kg	(16.5 % protein)
PI	30 Kg	(69.0 % protein)
SII	52 Kg	(4.2 % protein)
PII	18 Kg	(49.6 % protein)

Fig. 4. Flow sheet and yields of products produced by pin milling and air classification of dehulled faba-beans.

amino acid analyzer. Cystine was oxidized to cysteic acid and methionine converted to a sulfone derivative with performic acid before hydrolysis with hydrochloric acid according to Hirs (1967).

Analysis of variance was carried out according to Snedecor and Cochran (1973) and differences among treatment means were determined using Student-Newman-Keul's multiple range test as outlined by Kirk (1968) or where applicable using Student's "t" test (Snedecor and Cochran 1973).

PRODUCTIVE PERFORMANCE OF LAYING HENS FED FABABEAN CONTAINING DIETS

EXPERIMENT 1

Influence of Heat Treatment

Two trials were conducted to determine if heat treatment would alleviate the depressing effect of high levels of fababeans in the diets of laying hens on egg production and egg weight. In trial 1, two hundred and fifty-six (256) Hyline-W36 layers were housed in community pens equally subdivided into two cages (40 cm x 40 cm) with a common water fount and feeder. The birds were allocated randomly to 4 treatments at 44 weeks of age with 8 replicates of 8 hens each per treatment group. Treatments arranged in a completely randomised design consisted of a wheat-soybean based control diet and 3 wheat-fababean based diets in which fababeans constituted the sole source of

supplemental protein (Table 1). The control diet was fed in the mash form while the fababean diets were fed either as mash (diet 2) or pellets (diets 3 and 4). Prior to compounding of ingredients in diet 4, fababeans were extruded at 132°C (306°F) in a Brady Crop Cooker, Koehring. Pelleting was done at 70°C (150°F) in a commercial pelleter (California Master Pellet Mill). All diets were supplemented with DL-methionine to meet the requirements of the laying hen for methionine as listed by NRC (1971). Feed and water were offered ad libitum throughout the 140-day experimental period during which daily hen-day egg production, egg weight (obtained over 3 consecutive days at the termination of each of 5, 28-day periods), feed intake, mortality and initial and final body weight data were collected.

In trial 2, ninety-six (96) hens of each of two strains (Shaver-288 and Dekalb) housed as described in trial 1, were allocated randomly at 29 weeks of age to 6 treatments with 2 replicates of 8 hens from each strain per treatment. The composition of experimental diets, containing raw or autoclaved (121°C for 30 minutes) whole fababeans, dehulled fababeans, fababean hulls and starch formulated to be isonitrogenous and adequate in methionine is given in Table 2. Data collection was carried out for 5, 28-day periods as described for trial 1.

Table 1. Composition and Chemical Analysis of Diets (Experiment I, Trial 1)

Ingredients	Diets 1 %	2 %	3 %	4 %
Wheat	70.2	45.6	45.6	45.6
Soybean meal	16.4	-	-	-
Fababeans	-	40.0	40.0	40.0
Rapeseed oil	1.0	2.0	2.0	2.0
Mineral mix[1]	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0
Others[3]	10.9	10.9	10.9	10.9
Chemical Analyses[4]				
Protein (N x 6.25)	18.6±0.1	17.4±0.2	17.3±0.0	18.7±0.2
Fat (ether extract)	3.1±0.0	3.6±0.0	3.8±0.0	3.5±0.0
Crude fiber	3.1±0.0	5.2±0.0	5.1±0.0	5.1±0.0
Ash	9.1±0.1	9.1±0.1	9.4±0.0	9.2±0.0

[1]The composition of mineral mix (mg/kg diet) was: manganese, 330 as MnO; zinc, 110 as ZnO and iodized NaCl, 9650.

[2]The composition of the vitamin mix per kg diet was: retinyl palmitate (Vit. A), 8260 I.U.; cholecalciferol (Vit. D_3), 880 I.U.; α-tocopherol (Vit. E), 248 I.U.; Vit. B_{12}, 0.011 mg; riboflavin, 8.93 mg; calcium pantothenate (pantothenic acid), 17.86 mg; niacin, 26.79 mg; choline chloride, 446.43 mg; DL-methionine, 500 mg or as required to meet NRC (1971) requirements; anti-oxidant (santoquin), 250 mg; tallow, 100 mg and wheat middlings, 8430 mg.

[3]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2% and dehydrated alfalfa, 2.0%.

[4]Chemical analyses represent average ±S.E. for duplicate samples.

Table 2. Composition and Chemical Analysis of Diets (Experiment I, Trial 2)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %	6 %
Wheat	45.6	45.6	45.6	45.6	54.3	54.3
Soybean meal	-	-	-	-	19.3	19.3
Whole fababeans	40.0	-	-	-	-	-
Autoclaved fababeans	-	40.0	-	-	-	-
Dehulled fababeans	-	-	36.4	-	-	-
Autoclaved dehulled fababeans	-	-	-	36.4	-	-
Fababean starch	-	-	3.6	-	-	-
Autoclaved fababean starch	-	-	-	3.6	-	-
Fababean hulls	-	-	-	-	10.0	-
Autoclaved fababean hulls	-	-	-	-	-	10.0
Rapeseed oil	2.0	2.0	2.0	2.0	4.0	4.0
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Others[3]	10.9	10.9	10.9	10.9	10.9	10.9
Chemical Analyses[4]						
Protein (N x 6.25)	17.6±0.5	18.1±0.5	18.6±0.5	18.2±0.4	18.4±0.0	18.6±0.8
Fat (ether extract)	3.7±0.1	3.8±0.1	3.5±0.1	3.7±0.0	5.6±0.0	5.5±0.0
Crude fiber	5.3±0.4	5.0±0.2	2.6±0.2	2.7±0.1	6.9±0.0	7.2±0.3
Ash	9.1±0.1	10.8±0.0	10.1±0.8	9.9±0.6	9.5±0.1	9.9±0.6

[1,2,3,4]See footnotes 1, 2, 3 and 4 listed in Table 1.

EXPERIMENT II

Influence of Whole and Dehulled Fababeans Fed Over a 280-day Period

Six hundred and thirty (630) Shaver-288 layers housed in community pens equally subdivided into two cages (30 cm x 40 cm) and with a common water fount and feeder, were alloted randomly at 34 weeks of age to 7 treatments with 15 replicates of 6 hens each per treatment group. The treatments, arranged in a completely randomised design consisted of the following diets formulated as shown in Table 3: (1) Control, (2) 15% whole fababeans, (3) 30% whole fababeans, (4) 13.5% dehulled fababeans, (5) 27% dehulled fababeans, (6) low energy control (diet 1 diluted with alpha-floc to approximate the energy content of diet 3) and (7) high energy fababean diet (diet 3 fortified with tallow to approximate the energy content of diet 5). All contained adequate supplemental methionine according to NRC (1971).

Feed and water were provided ad libitum throughout the 280-day experimental period during which daily hen-day egg production, feed consumption, egg weight (obtained over 3 consecutive days at the end of each of 10, 28-day periods), mortality and initial and final body weight data were recorded. During the eighth 28-day period, five replicates from each treatment were selected at random for metabolizable energy (M.E.) determinations. Chromic oxide (0.2%) was

Table 3. Composition and Chemical Analysis of Diets (Experiment II)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %	6 %	7 %
Wheat	71.9	62.5	53.9	62.5	53.9	65.7	47.2
Soybean meal	12.0	6.4	-	6.4	-	14.2	2.2
Fishmeal	2.2	2.2	2.2	2.2	2.2	2.2	2.2
Tallow	1.5	1.5	1.5	1.5	1.5	0.5	6.0
Whole fababeans	-	15.0	30.0	-	-	-	30.0
Dehulled fababeans	-	-	-	13.5	27.0	-	-
Fababean Starch	-	-	-	1.5	3.0	-	-
Alpha-floc	-	-	-	-	-	5.0	-
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Others[3]	10.9	10.9	10.9	10.9	10.9	10.9	10.9
Chemical Analyses[4]							
Protein (N x 6.25)	17.5±0.0	17.8±0.0	17.8±0.0	17.9±0.2	17.8±0.0	17.3±0.3	17.9±0.1
Calcium	3.5	3.3	3.3	3.2	3.4	3.2	3.3
Phosphorus	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Corrected M.E.(kcal/kg)	2774	2739	2726	2674	2848	2639	2932
Calculated Analyses							
M.E. (kcal/kg)	2746	2631	2537	2708	2691	2535	2696

[1,2,3,4]See footnotes 1, 2, 3 and 4 listed in Table 1.

mixed thoroughly into a portion of each diet and this chromic oxide containing feed was offered to previously selected groups of hens. Excreta samples were collected daily for two consecutive days after a five day acclimatization period. The excreta samples were frozen immediately after collection and then lyophilized. Feed and dried excreta samples were ground to pass through a 1 mm screen. The two ground excreta samples from each pen were pooled and a representative sample was taken for chemical analyses. The ground feed and excreta samples were assayed for gross energy (G.E.) using a Parr Oxygen Bomb calorimeter. The feed and excreta chromic oxide concentrations were determined by the method of Czarnoski et al. (1961). The nitrogen content of the samples was measured by the procedures of the A.O.A.C. (1970). Classical M.E. values of the diets were determined by application of the following equation:

$$\text{M.E./gm. feed} = \text{G.E./gm. feed} - \left(\frac{\text{Cr}_2\text{O}_3\text{/gm. feed}}{\text{Cr}_2\text{O}_3\text{/gm. excreta}} \times \text{G.E./gm.excreta} \right)$$

Dietary M.E. values corrected to nitrogen equilibrium were obtained by application of the following equation.

$$\text{Corrected M.E./gm. feed} =$$

$$\text{Classical M.E.} - \text{G.N./gm. feed} - \left(\frac{\text{Cr}_2\text{O}_3\text{/gm. feed}}{\text{Cr}_2\text{O}_3\text{/gm. excreta}} \times \text{G.N./gm. excreta} \right)$$

where G.N. is the gross nitrogen expressed in grams.

EXPERIMENT III

Influence of Dietary Methionine Levels

Two hundred and sixty-four (264) Shaver-288 layers in community pens, equally subdivided into two cages (30 cm x 40 cm each) with a common water fount and feeder, were allotted at random at 42 weeks of age to 4 treatments with 11 replicates (pens) of 6 birds each per treatment group. The treatments arranged in a completely randomised design consisted of a control diet and three fababean containing diets supplemented with 0.05 (diet 2), 0.14 (diet 3) and 0.23% (diet 4) DL-methionine. The ingredient composition and proximate analyses of the diets is given in Table 4 and the amino acid content of the diets is shown in Table 5. Feed and water were provided ad libitum throughout the 196-day experimental period during which daily hen-day egg production, egg weight (average weights of eggs collected for 3 consecutive days at the end of each of 7, 28-day periods), feed consumption, mortality, and initial and final body weight data were recorded.

EGG WEIGHT DEPRESSING EFFECT OF FABABEANS WHEN FED TO LAYING HENS

EXPERIMENT IV

Influence of Dietary Energy and Protein Levels

Two trials were conducted to determine the influence of the two major dietary components, energy and protein on

Table 4. Composition and Chemical Analysis of Diets (Experiment III)

Ingredients	Diets 1	2	3	4
	%	%	%	%
Barley	71.1	53.9	53.8	53.7
Soybean meal	16.9	8.0	8.0	8.0
Tallow	1.7	2.8	2.8	2.8
Fababeans	-	25.0	25.0	25.0
Mineral mix[1]	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0
DL-methionine[3]	-	-	0.1	0.2
Others[4]	8.8	8.8	8.8	8.8
Chemical Analyses[5]				
Protein (N x 6.25)	15.9±0.3	15.6±0.3	15.8±0.1	16.2±0.4
Fat (ether extract)	2.0±0.3	2.2±0.4	3.0±0.2	2.4±0.0
Crude fiber	4.6±0.0	4.8±0.0	4.7±0.1	4.7±0.5

[1,2] See footnotes 1 and 2 under Table 1.

[3] DL-methionine was premixed in the normal laying hen vitamin mix before adding to the diets.

[4] Other ingredients included: calcium carbonate, 4.3%; oystershell, 2.0%; dicalcium phosphate, 1.5% and dehydrated alfalfa, 1.0%.

[5] Chemical analyses represent average ±S.E. for duplicate samples.

Table 5. Amino Acid Composition of Diets (Experiment III)

Amino acids[1]	Diets 1 %	2 %	3 %	4 %
Arginine	0.99±0.16	1.16±0.08	1.14±0.08	1.05±0.15
Lysine	0.79±0.04	0.81±0.03	0.83±0.03	0.78±0.09
Histidine	0.36±0.02	0.37±0.02	0.37±0.02	0.35±0.02
Isoleucine	0.64±0.01	0.65±0.03	0.66±0.04	0.61±0.04
Leucine	1.16±0.03	1.18±0.04	1.18±0.05	1.11±0.07
Methionine[2]	0.35±0.01	0.31±0.01	0.48±0.07	0.58±0.06
Cystine[2]	0.28±0.00	0.29±0.01	0.28±0.00	0.34±0.00
Phenylalanine	0.74±0.02	0.74±0.04	0.73±0.04	0.66±0.06
Tyrosine	0.37±0.02	0.36±0.02	0.37±0.02	0.35±0.04
Threonine	0.53±0.02	0.56±0.02	0.57±0.02	0.51±0.03
Valine	0.78±0.03	0.76±0.03	0.76±0.04	0.75±0.03

[1]Amino acid analyses results are based on average ±S.E. of triplicate samples.

[2]Methionine and cystine were determined by the method of Hirs (1967).

the egg weight depression caused in laying hens by feeding fababeans. In trial 1, six hundred and seventy-two (672) Hyline-W36 layers housed in community pens equally subdivided into two cages (30 cm x 40 cm) with a common water fount and feeder, were allotted at 46 weeks of age to 8 treatments with 14 replicates of 6 hens each per treatment group. The experimental design was completely randomised with a 2 x 4 factorial arrangement. The ingredient composition, protein and energy levels of the diets are shown in Table 6. The fababean and soybean meal diets calculated to be isocaloric, were varied in energy content by altering the amount of soybean oil added to the diets. No attempt was made to equalize the protein:calorie ratios among the diets differing in energy content although the highest energy diets were formulated to contain an adequate level of dietary protein.

Feed and water were offered ad libitum throughout the duration of the 32-day experimental period and feed consumption data was collected. The weight of eggs laid every second day was recorded.

In trial 2, the same groups of hens were used subsequent to the completion of the first trial. A similar 2 x 4 factorial arrangement was employed with the presence and absence of fababeans in the diet as one factor and the level of dietary protein as the other factor. The ingredient composition, protein and energy contents of the diets used are shown in Table 7. Fababean protein concentrate (FBPC)

Table 6. Composition and Chemical Analysis of Diets (Experiment IV, Trial 1)

Ingredients	Diets 1	2	3	4	5	6	7	8
	%	%	%	%	%	%	%	%
Barley	55.6	52.6	49.6	46.6	70.1	67.1	64.1	61.1
Fishmeal	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Soybean meal	-	-	-	-	13.9	13.9	13.9	13.9
Fababeans	30.0	30.0	30.0	30.0	-	-	-	-
Soybean oil	-	3.0	6.0	9.0	-	3.0	6.0	9.0
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Alpha-floc[3]	-	-	-	-	1.6	1.6	1.6	1.6
Others[4]	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9
Chemical Analysis[5] Protein (N x 6.25)	17.6	16.8	16.3	15.9	18.2	17.7	17.5	17.1
Calculated Analysis M.E. (kcal./kg)	2430	2620	2800	2980	2430	2620	2800	2980

[1,2]See footnotes 1 and 2 listed in Table 1.

[3]Alpha-floc was from Brown and Company.

[4]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0% and dicalcium phosphate, 2.2%.

[5]Chemical analysis results are based on single samples.

Table 7. Composition and Chemical Analysis of Diets (Experiment IV, Trial 2)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %	6 %	7 %	8 %
Barley	73.5	71.0	68.5	66.0	68.0	65.5	63.0	60.5
Soybean meal	10.1	10.1	10.1	10.1	-	-	-	-
Fishmeal	-	2.5	5.0	7.5	-	2.5	5.0	7.5
Soybean oil	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
FBPC[3]	-	-	-	-	10.0	10.0	10.0	10.0
Corn Starch	-	-	-	-	5.6	5.6	5.6	5.6
Others[4]	8.9	8.9	8.9	8.9	8.9	8.9	8.9	8.9
Chemical Analyses[5]								
Protein (N x 6.25)	15.1	16.5	18.5	19.8	15.1	17.2	18.5	20.1
Calculated Analyses								
Protein	14.0	15.4	17.0	18.4	14.4	15.9	17.4	18.9
M.E. (kcal /kg)	2855	2858	2860	2860	2900	2910	2910	2910

[1,2]See footnotes 1 and 2 listed in Table 1.

[3]Fababean protein concentrate.

[4,5]See footnotes 4 and 5 listed in Table 6.

was used to formulate the fababean diets and the varying protein levels in the diets were obtained by altering the content of fishmeal and barley. As in trial 1, feed and water were offered ad libitum throughout the 32-day experimental period and egg weight was recorded on alternate days.

EXPERIMENT V

A Study of the Feasibility of Utilizing a 14-day Test Period

A series of trials were conducted with Shaver-288 laying hens at 42 weeks of age to monitor the effects on egg weight of feeding ground fababeans and fababean protein concentrate (FBPC). The hens housed in individual cages (30 cm x 40 cm) were divided randomly into two groups of 25 hens each with one group designated as control group and the other as treatment. Control diets were fed to the control group at all times whereas fababean containing test diets were fed to the treatment group during 14-day test periods which were separated by a control period during which the control diet was re-fed. Diets fed were as presented in Table 8. The control group served to monitor the gradual change in egg weight as the hens became older while the treatment group served to monitor any additional changes in egg weight as a consequence of the hens being fed the various fababean containing diets. Eggs were collected and egg weight recorded daily. The effects of the treatments were evaluated by describing the egg weight

Table 8. Composition and Analysis of Diets (Experiment V)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %	6 %	7 %
Barley	63.7	-	-	73.0	73.0	68.3	68.0
Fababeans	-	84.0	79.0	-	-	-	-
Soybean meal	23.3	-	-	4.7	-	4.7	-
Fishmeal	-	-	-	5.3	-	10.0	5.0
Soybean oil	-	-	5.0	6.0	6.0	6.0	6.0
Rapeseed oil	-	5.0	5.0	-	-	-	-
Tallow	2.0	-	-	-	-	-	-
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
FBPC[3]	-	-	-	-	10.0	-	10.0
Others[4]	9.5	9.5	9.5	9.5	9.5	9.5	9.5
Calculated Analyses							
Protein (N x 6.25)	19.3	22.7	21.3	15.0	15.0	18.1	18.2
M.E. (kcal/kg)	2400	2500	2800	2880	2860	2880	2800

[1,2]See footnotes 1 and 2 listed in Table 1.

[3]Fababean protein concentrate.

[4]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2% and wheat middlings 0.6%.

data graphically.

THE USE OF SHORT DURATION TEST PERIODS TO STUDY THE EGG WEIGHT DEPRESSING EFFECT OF FABABEAN FRACTIONS AND TO ISOLATE THE EGG WEIGHT DEPRESSING FACTOR IN FABABEANS

EXPERIMENT VI

Influence of Autoclaving Treatment on the Egg Weight Depressing Effect of Fababean Protein Concentrate

A 14-day test procedure similar to that developed in Experiment V was utilized to determine if egg weight depressing effect of FBPC could be alleviated by heat treatment. The alterations to the technique were that the weight of the eggs laid during the initial control period was used to assign hens to control or treatment groups such that the mean egg weight of the groups was similar. In addition, statistical analysis of the egg weight data to complement the graphical presentation was used. The analysis involved a comparison between groups of the percent change in egg weight during the test period in relation to the preceeding control period.

Fababean protein concentrate (FBPC), prepared by air classification as described previously, was divided into two parts. One half was not subjected to heat treatment and was called untreated fababean protein concentrate (UFBPC). The second half, was spread in porcelain trays to a depth of 2 cm and was autoclaved at 121^{o}C for 30 minutes. The product was designated autoclaved fababean

protein concentrate (AFBPC). Both UFBPC and AFBPC were added to the respective diets at a level of 12.2%. Fishmeal (5.3%) was added to the diet that contained AFBPC to compensate for protein that may have been rendered unavailable to the bird by autoclaving. The specific protocol used in the experiment is outlined in Table 19 and the composition of the diets (1, 2 and 3) is shown in Table 9.

During the test period when the AFBPC containing diet was fed to the treatment group, eggs were collected over a three-day period. The eggs were broken out and the yolk was separated, weighed, lyophilized and re-weighed. The albumen was collected, weighed, dried in an oven and re-weighed. The shell was washed and allowed to dry overnight before weighing. The dried yolks from each treatment group were pooled and samples were taken for total lipid determinations.

EXPERIMENT VII

The Effect of Fababean Hulls on Egg Weight

The influence of feeding a diet that contained 10% fababean hulls to laying hens on egg weight was studied using the 14-day test procedure described in Experiment VI.

Fababean hulls were cleaned by sieving to eliminate cotyledons and other contaminants. The hulls were then ground before adding to the diet. The hull containing diet

Table 9. Composition and Chemical Analysis of Diets (Experiments VI, VII, VIII and IX)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %	6 %	7 %	8 %
Wheat	70.6	72.1	66.8	53.6	40.6	72.9	68.6	65.6
Fishmeal	5.9	-	5.3	5.9	8.0	-	5.9	5.9
Soybean meal	7.3	-	-	10.1	8.5	-	7.3	7.3
Fababean hulls	-	-	-	10.0	-	-	-	-
Fababean starch	-	-	-	-	26.0	-	-	-
Extract	-	-	-	-	-	-	2.0	5.0
Soybean oil	4.0	4.0	4.0	9.0	5.0	4.0	4.0	4.0
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
UFBPC[3]	-	12.2	-	-	-	-	-	-
AFBPC[4]	-	-	12.2	-	-	-	-	-
EFBPC[5]	-	-	-	-	-	11.4	-	-
Alpha-floc	0.8	0.3	0.3	-	0.5	0.3	0.8	0.8
Others[6]	9.9	9.9	9.9	9.9	9.9	9.9	9.9	9.9
Chemical Analysis Protein (N x 6.25)	17.5	17.2	19.7	17.3	17.1	17.4	17.6	17.7

[1,2] See footnotes 1 and 2 listed in Table 1.
[3] Untreated fababean protein concentrate.
[4] Autoclaved fababean protein concentrate.
[5] Extracted fababean protein concentrate.
[6] Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2% and dehydrated alfalfa, 1.0%.

was fortified with soybean oil to increase the energy density of the diet

Two groups of 20 Babcock laying hens each at 46 weeks of age were used. The specific regime followed in the experiment is given in Table 21 and the composition of the diets (1 and 4) is shown in Table 9.

EXPERIMENT VIII

The Influence of Fababean Starch and the Effect of Ethanol-Water Extraction of Fababean Protein Concentrate

An experiment was conducted to determine if fababean starch (FBS) would cause egg weight depression and to evaluate the influence of ethanol-water extraction on the egg weight depressing effects of FBPC utilizing the 14-day test procedure described in the previous experiments.

The FBS and FBPC used in this study were obtained as described earlier. The ethanol-water extract and EFBPC were prepared by adding 6 volumes of ethanol and water in the ratio of 60:40 to the FBPC (58% crude protein). The mixture was allowed to stand for 30 minutes at 20°C and was then homogenized using a Teckmar-SD45N homogenizer. The homogenate was centrifuged at 9,000 x g for 10 minutes in a refrigerated Sorvall RC2-B automatic centrifuge. The supernatant was decanted and saved. The partially extracted FBPC was re-extracted with 5 volumes of ethanol and water and the first and second supernatants were pooled and concentrated by evaporating ethanol and a portion of the

water in a cyclone evaporator. The concentrated extract and the EFBPC were then lyophilized. The yield of the dried extract was 14% while that of the dried EFBPC was 80% of the original FBPC. The protein (N x 6.25) content of the extract and the EFBPC was 28 and 64%, respectively.

The specific regime followed in the experiment is given in Table 22 and the composition of the diets (1, 2,5,6 and 7) is shown in Table 9. In this experiment 3 groups (1 control and 2 treatment) each of 30 Babcock-B300 layers at 41 weeks of age were used. The two treatment groups were fed diets that contained either FBS (diet 5) or an ethanol-water extract of FBPC (diet 7) during the first test period and diets that contained either FBPC (diet 2) or ethanol-water extracted FBPC (diet 6), respectively, during the second test period.

EXPERIMENT IX

Further Studies with Ethanol-Water Extract of Fababean Protein Concentrate

A bioassay, similar to the 14-day test procedure described in previous experiments, was used to determine the effect on egg weight of layer diets containing varied levels of an ethanol-water extract of FBPC.

In this study, 3 groups of 30 Shaver-288 layers each at 26 weeks of age were involved in a 7-day test period to monitor the effects of feeding 2 and 5% ethanol-water extract prepared from FBPC as described in Experiment

VIII. The specific regime followed in the experiment is given in Table 23 and the composition of the diets (diets 1, 7 and 8) is shown in Table 9.

EXPERIMENT X

The Influence of pH Fractionation on an Ethanol-water Extract of Autoclaved Fababean Protein Concentrate

An experiment was conducted utilizing the 7-day test procedure adapted in Experiment IX to establish the effect of pH fractionation of an ethanol-water extract of AFBPC on egg weight.

The method for preparing the ethanol-water extract was modified. Fababean protein concentrate (FBPC) was autoclaved at 121°C for 10 minutes. The AFBPC was then extracted with 8 volumes of ethanol and water in the ratio of 1:1. The mixture was homogenized as before and was passed through two layers of cheesecloth. The insoluble residue was discarded. The filtrate was allowed to stand overnight to permit sedimentation of particulate matter that passed through the cheesecloth. Approximately 80% of the relatively clear filtrate was then removed by siphoning. The remaining filtrate was centrifuged at 36,000 x g for 10 minutes. The two supernatants were pooled and concentrated by evaporating the ethanol and a portion of the water in a cyclone evaporator. The concentrated extract was lyophilized and kept at -20°C until fractionated. The yield of the dry extract was 10 to 11% of the original AFBPC. The protein

content was 29%.

The lyophilized extract of AFBPC was dissolved in water in the ratio of 1 g of extract to 10 ml of distilled water (1:10) and the pH was adjusted to 4 using 2N HCl. The mixture was centrifuged at 36,000 x g for 10 minutes. The supernatant was decanted, the pH further reduced to 3 using 2N HCl and centrifuged as before. Supernatant and precipitate fractions were separated and saved. The pH 4 precipitate was washed with 10 volumes of water and centrifuged as described previously. The washings were pooled with the pH 3 supernatant while the washed pH 4 precipitate was pooled with the pH 3 precipitate. The pooled supernatant was concentrated by evaporating a fraction of the water in a cyclone evaporator. The supernatant and the precipitate were then lyophilized. The percent dry matter and crude protein of the soluble fraction and precipitate were 91 and 27; 98 and 33, respectively. The soluble and precipitate fractions were added to the diets at 4.6 and 0.7% levels, respectively.

Three groups of 30 Shaver-288 hens each at 35 weeks of age were used. The specific regime followed in the experiment is given in Table 24 and the composition of the diets is shown in Table 10.

Table 10. Composition of Diets (Experiment X)

Ingredients	Diets 1 %	2 %	3 %
Wheat	70.6	69.9	66.0
Fishmeal	5.9	5.9	5.9
Soybean meal	7.3	7.3	7.3
Precipitate	-	0.7	-
Supernatant	-	-	4.6
Soybean oil	4.0	4.0	4.0
Mineral mix[1]	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0
Others[3]	10.7	10.7	10.7

[1,2]See footnotes 1 and 2 under Table 1.

[3]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2%; dehydrated alfalfa, 1.0% and alpha-floc (from Brown and Co.), 0.8%.

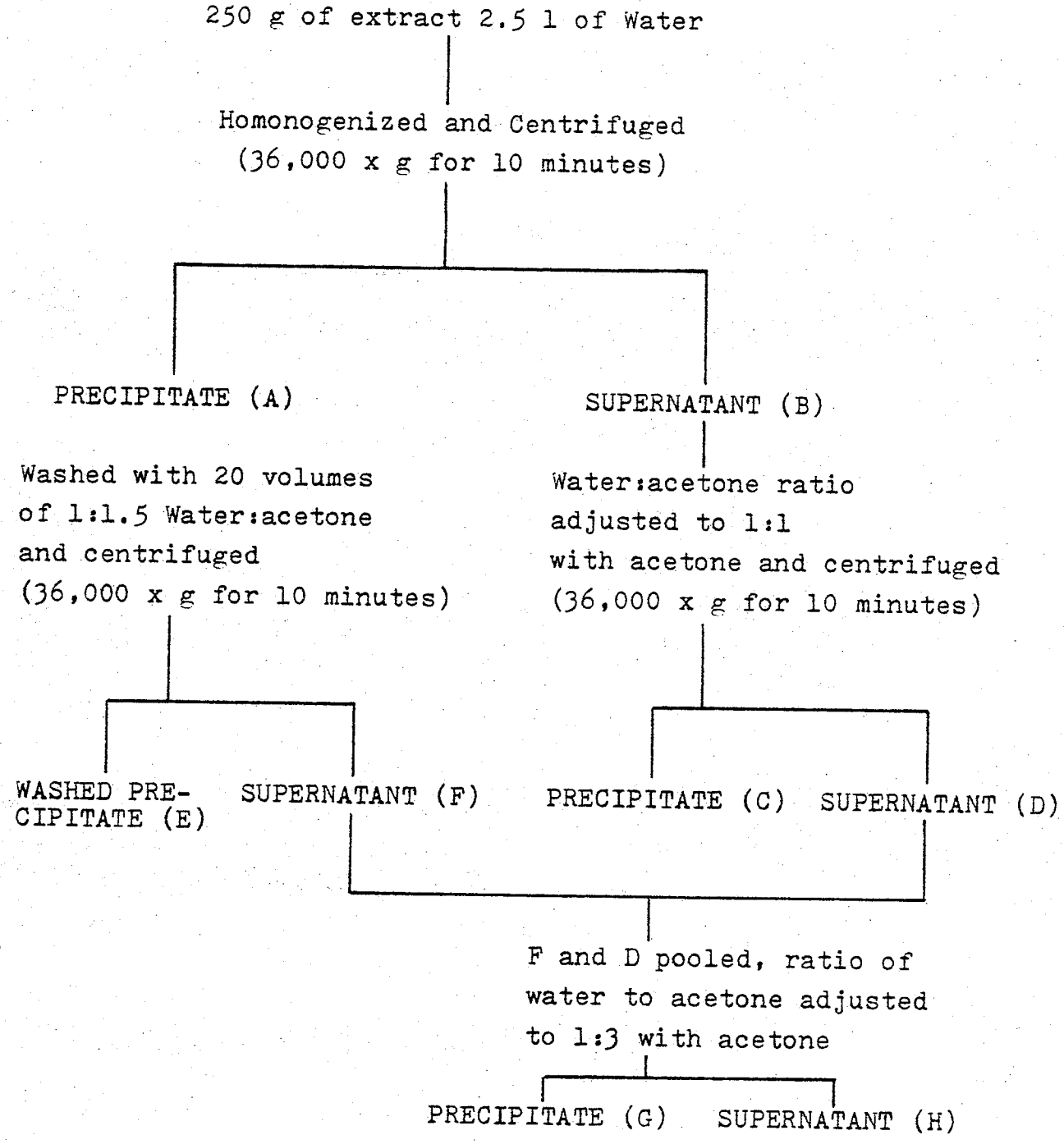
EXPERIMENT XI

The Influence of Acetone Fractionation on an Ethanol-water Extract of Autoclaved Fababean Protein Concentrate

The ethanol-water extract of AFBPC was further fractionated into four fractions using acetone. The fractions were fed to laying hens using the 7-day test procedure to determine the egg weight depressing activity of each fraction.

The ethanol-water extract, prepared as described in Experiment X, was dissolved in distilled water in the ratio of 1 g of extract to 10 ml water (1:10). The mixture was centrifuged at 36,000 x g for 10 minutes. This produced two fractions, one insoluble in water (A) and the other soluble (B) (Fig. 5). Acetone was added to the soluble fraction (B) so that the ratio of water to acetone was 1:1. The mixture was agitated for 5 minutes and then centrifuged at 36,000 x g for 10 minutes to separate the precipitate (C) and supernatant (D). The original water insoluble fraction (A) was washed with 20 volumes of water and acetone in the ratio of 1:1.5 and the insoluble fraction (E) was saved. The washings (F) were pooled with the supernatant D. Acetone was added to the pooled water-acetone soluble fractions (D and F) so that the final water to acetone ratio was 1:3. The mixture was stirred and allowed to stand for 30 minutes. This resulted in two fractions, an insoluble fraction (G) which precipitated out, and a soluble fraction (H) which was decanted and concentrated in a cyclone

Fig. 5. Water:Acetone Fractionation Procedure



evaporator. The washed water insoluble fraction (E), the 1:1 water:acetone insoluble fraction (C), the 1:3 water:acetone insoluble fraction (G) and the concentrated 1:3 water:acetone soluble fraction (H) were lyophilized. The yields of the various fractions were 5.5, 9.4, 48.1 and 31.3% of the original ethanol-water extract, respectively.

The washed water insoluble fraction (E) and the 1:1 water:acetone insoluble fraction (C) were pooled and fed as one sample. The various fractions were added to the respective diets according to their proportions in the ethanol-water extract. Amino acids in the proportions and amounts equivalent to those in the extract were premixed in corn starch before they were added to the diet.

Six groups of 20 Shaver-288 laying hens each at 26 weeks of age were involved. The specific protocol followed in the experiment is given in Table 25 and the composition of the diets is shown in Table 11.

At the end of the test period, 5 hens from each group were selected at random, killed by cervical dislocation and the livers were immediately excised, placed in plastic bags and weighed. The livers were then lyophilized, ground individually and samples of each were taken for total lipid determination.

Table 11. Composition of Diets (Experiment XI)

Ingredients	Diets					
	1	2	3	4	5	6
	%	%	%	%	%	%
Wheat	70.6	60.4	60.4	68.9	65.0	67.0
Fishmeal	5.9	5.9	5.9	5.9	5.9	5.9
Soybean meal	7.3	7.3	7.3	7.3	7.3	7.3
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0	1.0
Amino acid mix[3]	-	5.7	-	-	-	-
Extract	-	-	11.0	-	-	-
1:1 Water:acetone fraction[4]	-	-	-	1.7	-	-
1:3 Water:acetone fraction[5]	-	-	-	-	5.6	-
Supernatant-H	-	-	-	-	-	3.6
Alpha-floc	0.8	5.3	-	0.8	0.8	0.8
Others[6]	13.9	13.9	13.9	13.9	13.9	13.9

[1,2]See footnotes 1 and 2 listed in Table 1.

[3]Amino acid mix was composed of synthetic amino acids (1.7%) in amounts equivalent to those contributed by the extract in diet 3, premixed in corn starch (4.0%).

[4]1:1 water:acetone insoluble fraction (C) and water insoluble fraction (E).

[5]1:3 water:acetone insoluble fraction (G).

[6]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2%; soybean oil, 4.0% and dehydrated alfalfa, 1.0%.

EXPERIMENT XII

Isolation, Purification and Identification of the Egg Weight Depressing Factor

Fractionation of Supernatant-H

Previous experiments had demonstrated that supernatant-H, a water-acetone soluble fraction of an ethanol-water extract of AFBPC, contained a high activity of the egg weight depressing factor. Water solubility of supernatant-H was used to further fractionate this material in an attempt to obtain a relatively pure fraction of the egg weight depressing factor for use in feeding studies.

The solubility characteristics of supernatant-H in water were established at room temperature (26°C) using two methods. In the first method 10, 1 g samples of the dried supernatant-H were weighed into pre-weighed centrifuge tubes. Varying quantities (2, 4, 8, 16 and 32 ml) of water were added to the various tubes in duplicate sets. The solutions were vortexed every 10 minutes for 50 minutes and subsequently centrifuged at 27,000 x g for 10 minutes. The supernatant was decanted and the pH measured. The supernatant was then lyophilized. The tubes containing the precipitates were weighed after drying in an oven at 44°C for 21 hours. The dried precipitates were washed twice with 10 ml of acetone with centrifuging as before. The supernatants were decanted and discarded, while the precipitates were dried at 44°C for 40 minutes and the

weight of dry material recorded.

In the second method, samples of supernatant-H were first extracted with 10 volumes of acetone, and the resulting supernatants were decanted while the precipitates were dried in an oven at 44°C for 1½ hours. These dried precipitates were then treated with water as in the first method.

Based on the water solubility characteristics determined for supernatant-H, a fractionation procedure was developed to obtain soluble and insoluble fractions for further studies of the egg weight depressing factor. In this fractionation, supernatant-H was dissolved in water in the ratio of 1 g to 8 ml (1:8) with constant stirring for 30 minutes at room temperature (26°C). The mixture was centrifuged at 27,000 x *g* for 10 minutes. The precipitate was re-extracted with 0.6 volume of water (based on original weight of starting material) with constant stirring for 30 minutes and was centrifuged at 27,000 x *g* for 10 minutes. It was then washed twice with 1 volume of acetone (based on original weight of the starting material) with constant stirring for 5 minutes. The relatively white precipitate was collected following centrifugation at 3,000 x g for 10 minutes. An additional white precipitate formed when the two acetone soluble fractions were allowed to stand at 2°C for 12 hours. This precipitate which was equivalent to only 0.3% of the original starting material was harvested

by decanting and centrifugation and was pooled with the original white precipitate before drying at 44°C for 21 hours. The two water soluble fractions were also pooled and lyophilized. The acetone soluble fraction was evaporated at room temperature and the residue was pooled with the water soluble fraction. The two fractions thus obtained are subsequently referred to as white precipitate (pellet) and soluble fraction (supernatant).

Routine fractionations were generally initiated with 250 g of supernatant-H. The total amount of supernatant-H that was fractionated for the feeding trial was 1.35 kg. The amounts of the two fractions added to the test diets were 0.5% white precipitate and 3.7% soluble fraction. The total percent of the two fractions added to the diets (4.2) was slightly more than the concentration (3.6%) of supernatant-H used in Experiment XI. In addition to the diets containing the supernatant-H fractions, a diet containing a mixture of synthetic amino acids similar to the amino acid profile of supernatant-H and a diet containing L-dihydroxyphenylalanine (L-DOPA) at a concentration 6 times that found in supernatant-H were fed in the 7-day biological test trial.

Five groups of 24 Shaver-288 hens each at 32 weeks of age were used as the test birds. The specific regime used in the trial is given in Table 29 and the composition of the diets is shown in Table 12.

Table 12. Composition of Diets (Experiment XII)

Ingredients	Diets 1 %	2 %	3 %	4 %	5 %
Wheat	70.6	68.7	70.5	70.1	66.9
Fishmeal	5.9	5.9	5.9	5.9	5.9
Soybean meal	7.3	7.3	7.3	7.3	7.3
Mineral mix[1]	0.5	0.5	0.5	0.5	0.5
Vitamin mix[2]	1.0	1.0	1.0	1.0	1.0
Amino acid mix[3]	-	1.9	-	-	-
L-DOPA[4]	-	-	0.1	-	-
White precipitate	-	-	-	0.5	-
Supernatant	-	-	-	-	3.7
Others[5]	14.7	14.7	14.7	14.7	14.7

[1,2]See footnotes 1 and 2 listed in Table 1.

[3]Amino acid mix was composed of synthetic amino acids (1.3%) in amounts equivalent to those that would be contributed by supernatant-H if fed at 4.2% of diet, premixed in 0.6% wheat middlings.

[4]L-dihydroxyphenylalanine.

[5]Other ingredients included: calcium carbonate, 4.7%; oystershell, 2.0%; dicalcium phosphate, 2.2%; soybean oil, 4.0%; alpha-floc, 0.8% and dehydrated alfalfa 1.0%.

Purification of the White Precipitate

The white precipitate which appeared fairly pure was shown to contain a high activity of the alleged egg weight depressing factor. Preliminary studies were carried out to determine the influence of temperature and pH on the solubility characteristics of the white precipitate in an attempt to prepare an analytically pure egg weight depressing factor.

The initial procedure for studying the effect of temperature on water solubility involved a series of successive extractions of the same white precipitate sample at increasing temperatures. In the first series of extractions 0.66 g of white precipitate was added to 3 ml of water but in all subsequent extractions a volume of 6 ml was used. In all cases an excessive amount of undissolved precipitate was present and to use a minimum amount of sample the portion of each sample extracted at a given temperature was added back to the insoluble precipitate for further extractions.

The extractions were carried out by constant stirring for 30 minutes at a given temperature. The suspensions were then allowed to settle for 5 minutes before centrifuging at 15,000 x g for 5 minutes. These latter two steps were carried out at the same temperature as for the respective extractions except for the sample extracted at 80°C in which case the maximum centrifuge temperature

obtainable was 45°C. In all cases (except for the 80°C sample) the rotor of the centrifuge was pre-equilibriated to the extraction temperature. After centrifugation the colorless, aqueous supernatant was diluted and the optical density at 280 mu recorded. The temperature (±0.5°C) and dilutions involved were: 4°C and 225; 20°C and 450; 40°C and 900; 80°C and 900, respectively. All extractions were carried out in duplicate and the pH was determined to be 5.1.

To study the influence of pH on solubility 0.5 g of white precipitate was dissolved in 3 ml of water and allowed to stand at room temperature (26°C) for 30 minutes before being centrifuged at 15,000 x g for 5 minutes (at 20 to 25°C). The supernatant was decanted and the pH and optical density at 280 mu (450 fold dilution) were determined. The supernatant was recombined with the white precipitate using a vortex and the pH of this suspension was adjusted over a series of recombinations with either 0.4N HCl or 0.5N KOH to yield four pH ranges: 2.0 to 3.0; 4.0 to 4.5; 7.5 to 8.0 and 9.5 to 10.0. The solubility was determined for duplicate suspensions at each pH range by following the procedure outlined above except that 20 minutes rather than 30 minutes were allowed for the solubilization step.

After several preliminary trials, the following procedure was adopted for the preparation of analytically pure crystalline material from the white precipitate.

The white precipitate was dissolved in water in the ratio of 1 g to 20 ml. The pH was adjusted to between 9 and 10 using 3N NaOH and the suspension was heated to 80°C. It was then stirred at 80°C for 20 minutes with the occasional addition of NaOH so as to maintain a constant pH. The mixture was centrifuged at 15,000 x g for 10 minutes at 45°C, the supernatant was decanted, the pH was determined and the sample was allowed to stand overnight at 2°C which resulted in the production of copious amounts of crystalline material. The following day, the crystals were harvested from the supernatant by centrifuging at 15,000 x g for 20 minutes. The supernatant was decanted and saved. The crystals were washed twice with 5 volumes of cold water, once with 3 volumes of ethanol and once with 5 volumes of acetone with centrifuging after each washing. The product was allowed to stand at room temperature to evaporate the volatile solvents and was then lyophilized. All supernatant fractions were pooled and lyophilized.

Identification of the Egg Weight Depressing Factor

Analytically pure crystals were prepared from the white precipitate as described in the previous section. These crystals were subjected to microscopic observation, melting point determination, ultraviolet absorption spectra determination and column chromatography for the identification of the alleged egg weight depressing factor.

Microscopic observation of the crystalline compound was performed under two different instruments. In the first, the crystals which were suspended in a water solution were mounted on slides and observed under bright-field objectives with a Zeiss 64012 photomicroscope. Pictures of representative areas were taken at different magnifications. In the second observation the crystalline suspension was transferred by pasteur pipettes to an aluminum stub, allowed to air dry and subsequently coated with 1,000 A of gold in a Balzer Union Sputter Coater. Samples were then scanned in a Cambridge Stereo Scan Mark II at 10 KV. Photographs of representative areas were taken.

The melting point of the crystals was determined as described by Vogel (1956). A capillary tube filled with the finely ground crystals to a depth of 2 to 3 mm was immersed with a thermometer attached into a bath of medicinal paraffin. The melting point apparatus was heated with a low gas flame and the temperatures at which the sample commenced to melt and at which it completely decomposed were recorded.

The absorbance spectra of the crystalline substance were determined in a Unicam Spectrophotometer. Fifty milligrams of the crystals were dissolved in 100 ml of water. One milliliter of this solution was diluted to 15 ml with 0.1N HCl, 0.1N NaOH or 0.1M phosphate buffer (pH 6.8) and scanned against the appropriate blank (1 ml of water plus 14 ml of the diluting solution).

Column chromatography of four fababean fractions was carried out to test the purity and determine elution patterns of the alleged egg weight depressing factors. The samples were analysed in the long column (0.9 x 55 cm) of a Beckman model 119C automatic amino acid analyzer followed by detection of the products using ultraviolet absorption at 280 mu in Isco model UA-5 absorbance monitor. The column contained the Beckman W-1 resin which is a strong sulfonic acid cation exchanger being a copolymer product of styrene and divinylene. The elution buffers used were 0.2N sodium citrate (pH 3.25) and 0.2N sodium citrate (pH 4.12). The column temperature was 50°C. Buffer changes occurred at 40 and 60 minutes, respectively, after the initiation of the run.

Samples of the white precipitate (25.9 mg), crystals obtained from the white precipitate (20.5 mg) and the non-crystalline fraction (residue) (34.5 mg) were diluted to 100 ml with 0.2N sodium citrate buffer (pH 3.25). One gram of fababean protein concentrate was added to 10 ml of water. One milliliter of 0.67N H_2SO_4 and 1 ml of 10% sodium tungstate ($Na_2 WO_4.2H_2O$) were added to 2 ml of the solution. (Sulfuric acid and sodium tungstate interact to produce tungstic acid ($H_2 WO_4$) which is a deproteinizing agent) (Oser 1965). The solution was centrifuged at 25,000 x g for 20 minutes and the supernatant was decanted and re-centrifuged. One quarter of a milliliter of each

sample was added to the column. The column was washed with 0.2N NaOH after each run. All samples were analysed in duplicate and care was taken to dilute the samples with buffer pH 3.25 just immediately prior to column application to minimise possible hydrolysis of the compounds at the low pH.

The ultraviolet absorbing amino acids, L-DOPA, tyrosine and tryptophan were also chromatogramed. Buffer changes in this case occurred at 100 minutes.

EXPERIMENT XIII

A Comparison of Chemical Analysis of Total Vicine[1] in Various Fababean Fractions with the Observed Egg Weight Depressing Effects of these Fractions

A quantitative estimation of total vicine in various fababean fractions was carried out utilizing the methods of Higazi and Read (1974) and Collier (1976); and a column chromatographic method in order to compare the amounts of vicine in these fractions with their observed biologically determined egg weight depressing effects.

Two samples of 100 mg each of vicine crystals obtained as described previously were each dissolved in 10 ml of 10% trichloroacetic acid (TCA). Volumes of 0, 0.5, 1.0, 1.5, 2.0 and 3.0 ml which represented 0, 5, 10,

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[1] Also referred to as total ultraviolet absorbing material, total vicine reacting material or vicine and convicine.

15, 20 and 30 mg vicine were prepared. A vicine standard curve was established from the average OD^{650} values (Higazi and Read 1974). The above procedure was repeated with the white precipitate. Other fababean fractions were dissolved in 10 ml of 10% trichloroacetic acid and OD^{650} was recorded in order to estimate their vicine contents using the vicine standard curve. The quantities of samples used were 27.5, 35.0 and 51.2 mg for supernatant-H while 1 g sample of fababean hulls, fababean starch, untreated fababean protein concentrate and autoclaved fababean protein concentrate were used.

A method similar to that reported by Collier (1976) was also used to determine vicine content of various fababean fractions. For the analysis, specific weights (as listed above) of the different fractions were dissolved in 10 ml of 3% (W/V) metaphosphoric acid (HPO_3). The samples were allowed to stand for 15 minutes with constant mixing and were then centrifuged at 27,000 x g for 10 minutes. Supernatants were decanted and optical density at 280 mu was determined after diluting with 0.1N HCl.

In addition to the two established methods, a column chromatographic procedure was developed to quantitate vicine in the various fractions. In this procedure, pieces of paper representing the convicine and vicine peaks on the chromatograms established in Experiment XII were cut out and weighed to determine the areas of the convicine and vicine peaks. The amount of vicine in the crystalline

material was quantitated as follows:

Weight of area of convicine peak = 1.70 mg

Weight of area of vicine peak = 39.65 mg

Total area of convicine + vicine peak = 41.35 mg

Percent vicine in crystalline material

$$= \frac{\text{weight of area of vicine peak}}{\text{weight of area of convicine + vicine peaks}} \times 100$$

$$= \frac{39.65}{41.35} \times 100 = 96.0$$

The percent vicine and total vicine (convicine + vicine) in the various fababean fractions were calculated using the following procedure:

(i) Based on the above analysis, a standard sample (20.02 mg) of crystalline material was considered to contain 19.22 mg of vicine (20.02 x 0.96).

(ii) Amount of vicine per unit area of the vicine peak

$$= \frac{1}{39.65} \times 19.22 = 0.48 \text{ mg}$$

(iii) Amount of total vicine per unit area of the convicine + vicine peaks

$$= \frac{1}{41.35} \times 19.22 = 0.46 \text{ mg}$$

(iv) Percent vicine in an unknown sample

$$= \frac{\text{weight of vicine peak in unknown sample x (ii) x 100}}{\text{weight of unknown sample}}$$

(v) Percent total vicine in unknown sample

$$= \frac{\text{weight of total vicine peak in unknown sample x (iii) x 100}}{\text{weight of unknown sample}}$$

RESULTS AND DISCUSSION

PRODUCTIVE PERFORMANCE OF LAYING HENS FED FABABEAN CONTAINING DIETS

EXPERIMENT I

Influence of Heat Treatment

Trial 1

Extruding and Pelleting

The influence of heat treatment (steam pelleting or extruding) of fababeans on the productive performance of laying hens fed high levels of fababeans (40% of the diet) is shown in Table 13. Egg production was not significantly ($P>0.05$) affected by dietary treatments, although birds fed the fababean containing diets tended to have a lower percent hen-day production than the control birds. Steam pelleting or extruding did not alleviate the effect. A consistent but non-significant ($P>0.05$) egg weight depression was observed with the diets containing fababeans irrespective of heat treatment. Total egg mass, grams per hen per day, produced by birds fed the control diet was significantly ($P<0.05$) greater than that of birds given diets containing fababeans. Control birds produced an egg mass 11.5% greater than that produced by hens fed fababeans. The inclusion of 40% raw or heat treated fababeans into laying hen diets did not ($P>0.05$) have any influence on feed intake. Fababean containing diets were less efficiently ($P<0.05$) utilized than was the control diet, and since no

Table 13. Influence of Pelleting and Extruding of Fababeans on the Productive Performance of Laying Hens (Experiment I, Trial I)

Treatments	Egg production (% hen day)	Egg weight (g)	Total egg mass (g/h/d)	Feed intake (g/h/d)	Feed efficiency	Body weight gain (g)	Mortality %
Control (mash)	78.3±2.4[1]	56.4±0.6	44.2±1.4[a]	94.4±2.1	2.1±0.04[a]	193±15	2.3
Ground fababeans (mash)	70.5±3.2	54.5±0.3	38.4±1.9[b]	93.0±3.0	2.4±0.06[b]	191±20	2.3
Ground fababeans (pelleted)	71.7±2.2	54.8±0.6	39.3±1.2[b]	92.7±1.1	2.4±0.06[b]	203±18	2.7
Extruded fababeans (pelleted)	72.7±1.7	54.6±0.6	39.7±1.0[b]	92.3±1.5	2.3±0.03[b]	235±20	1.2

[1] Means ±S.E. not followed by the same superscript letter within a column are significantly different at $P<0.05$.

difference ($P>0.05$) was observed among the fababean diets, it can be concluded that heat treatment had no effect on the utilization of fababeans by laying hens. Weight gain of birds was not significantly ($P>0.05$) influenced by dietary treatments although it was higher with the extruded and pelleted fababean diets. Mortality which was at a low rate was not related to any specific dietary treatment.

These results would indicate that the nutritive value of fababeans for laying hens is not influenced by the heat generated during pelleting or extruding. On the contrary Davidson (1973), reported that pelleting fababean diets did have a beneficial effect on laying hen performance, however, the benefit was attributed to the improved feed intake. Davidson speculated that the heat generated during the pelleting process modified some deleterious factor(s) in the bean. Similarly, Marquardt et al. (1976), suggested that pelleting fababean diets resulted in improved chick performance due to a response in feed consumption. The change in the physical form of the feed resulting in a greater density was put forth as the explanation for the feed intake response rather than heat treatment per se. However, the same workers demonstrated that heat treatment at temperatures above that generated during steam pelleting also caused an improvement in the utilization of fababeans by chicks. This improvement was attributed to increased availability of nutrients as a result of the destruction of some toxic substance(s) or inhibitor(s) naturally present in fababeans. The results of the present experiment suggest

that the factor(s) that depress the performance of laying hens fed fababean containing diets are heat stable, and independent of those that affect the performance of chicks.

Trial 2

Autoclaving

The influence of autoclaving at 121°C for 30 minutes of whole fababeans, dehulled fababeans or fababean hulls on the productive performance of laying hens fed diets containing relatively high levels of these fababean components is summarized in Table 14. Except for egg weight, there were no significant ($P>0.05$) differences among any of the treatment means for egg production, total egg mass, feed consumption, feed efficiency or body weight gain. Egg weight although not significantly ($P>0.05$) affected by heat treatment, was influenced by the type of fababeans fed to the laying hens. Egg weight of birds fed whole or dehulled fababeans was 5.6 and 6.0% ($P<0.01$) lower than that of birds fed diets containing fababean hulls. Mortality was normal in all diets.

Whole fababeans contain 13% hulls (Marquardt et al. 1975), and consequently the hull content of the diet containing 40% whole fababeans can be calculated to be 5%. In this regard, since diets containing 10% hulls were associated with a significantly higher egg weight than the whole fababean diet, it can be suggested that fababean hulls per se are not responsible for the egg weight depressing effect

Table 14. Influence of Autoclaving on the Productive Performance of Laying Hens Fed Whole Fababeans, Dehulled Fababeans and Fababean Hulls (Experiment I, Trial 2)

Treatments	Egg production (% hen day)	Egg weight (g)	Total egg mass (g/h/d)	Feed intake (g/h/d)	Feed efficiency	Weight gain (g)	Mortality %
Raw whole beans (40%)	79.5±5.9[1]	57.1±0.8[B]	45.4±3.2	106.8±5.2	2.4±0.1	65.0±25.3	1.6
Autoclaved whole beans (40%)	82.8±1.7	56.6±0.4[B]	46.9±1.2	109.1±1.5	2.3±0.0	75.0±40.9	4.7
Raw dehulled beans (40%)	82.7±3.3	57.3±0.6[B]	47.5±2.4	104.2±4.2	2.2±0.1	150.0±45.2	3.7
Autoclaved dehulled beans (40%)	81.4±3.1	56.0±0.4[B]	45.6±1.6	101.0±1.1	2.2±0.1	232.5±44.4	2.1
Raw hulls (10%)	82.5±3.6	60.5±0.3[A]	49.9±2.0	113.1±0.6	2.3±0.1	180.0±54.9	0.5
Autoclaved hulls (10%)	82.6±2.0	60.0±0.6[A]	49.6±1.6	109.6±2.1	2.2±0.0	150.0±45.0	1.6

[1]Means ±S.E. not followed by the same superscript letter within a column are significantly different at $P<0.01$.

of whole fababeans. The egg weight depressing factor, therefore, appears to be concentrated mainly in the cotyledon as compared to the hull (testa) portion of the bean. These data also confirm the results reported in trial 1 of this experiment, that the egg weight depressing factor is heat stable.

EXPERIMENT II

Influence of Whole and Dehulled Fababeans Fed Over a 280-day Production Period

The effects of dietary treatments on egg production, egg weight, total egg mass, feed consumption, feed efficiency, energy intake, body weight gain and mortality are presented in Table 15. Although egg production was similar ($P>0.05$) for all treatment groups, the calculation of total egg mass revealed a lower ($P<0.05$) production by fababean fed hens as compared with control hens except for those hens which were fed the 15% whole fababean diet. This response was caused primarily by the progressive decrease in egg weight as the level of fababeans in the diet increased. Egg weight of hens fed the high energy control diet was not significantly ($P>0.01$) different from that of hens fed the 15% whole or dehulled fababean diets but was ($P<0.01$) different from that of hens which received diets containing 30% fababeans as whole, dehulled or whole made isocaloric to dehulled fababeans. Egg weight of hens fed the low energy control diet was significantly ($P<0.01$)

Table 15. The Productive Performance of Laying Hens Fed Whole and Dehulled Fababeans Over a 280-day Period (Experiment II)

Treatment		Egg production	Egg weight	Total egg mass	Feed consumption	Feed efficiency	Energy intake	Weight gain	Mortality
	(kcal M.E./kg)	(% hen day)	(g)	(g/h/d)	(g/h/d)		(kcal/h/d)	(g)	%
Control	(2774)	80.4±1.5[1]	62.2±0.3^{AB}	50.0±0.9^{ab}	108.9±1.6^{BC}	2.2±0.0^{a}	302.2±4.5^{A}	321.1±22.1	0.3
Control - low energy	(2639)	81.0±1.0	62.8±0.2^{A}	50.8±0.6^{a}	113.7±0.8^{A}	2.2±0.0^{a}	300.1±2.3^{A}	239.7±17.7	0.6
15% Whole fababeans	(2739)	80.3±1.5	60.7±0.4^{B}	48.8±0.8^{ab}	107.9±0.8^{BC}	2.2±0.0^{a}	295.7±2.3^{A}	275.1±17.2	0.5
15% Dehulled fababeans	(2674)	78.7±1.2	61.0±0.4^{B}	48.0±0.9^{b}	104.3±1.2^{C}	2.2±0.0^{a}	279.0±3.1^{B}	275.1±17.5	0.5
30% Whole fababeans	(2726)	79.9±1.2	59.9±0.4^{C}	47.8±0.7^{b}	110.4±0.6^{AB}	2.3±0.0^{b}	300.9±1.7^{A}	291.2±17.8	0.3
30% Dehulled fababeans	(2848)	79.8±1.1	59.5±0.4^{C}	47.5±0.7^{b}	104.3±1.3^{C}	2.2±0.0^{a}	297.0±3.8^{A}	287.8±12.9	0.5
30% Whole fababeans - High energy	(2932)	79.6±0.7	59.7±0.4^{C}	47.6±0.5^{b}	103.9±1.0^{C}	2.2±0.0^{a}	304.6±3.0^{A}	278.8±23.6	0.6

[1] Means ±S.E. not followed by the same superscript letter within a column are significantly different at ab = $P<0.05$ and ABC = $P<0.01$

greater than that of hens fed each of the fababean diets. The egg weight depression was consistent throughout the experiment (Fig. 6).

Dietary energy content was not a causative factor in the apparent reduction in egg mass as a consequence of feeding fababeans. Both the addition of tallow to the whole fababean diet or the dilution of the energy content of the control diet by addition of alpha-floc did not alter ($P>0.05$) the response noted. In fact, the hens were able to regulate energy intake whether fababeans were present in the diet or not as shown by the significant ($P<0.01$) differences in feed consumption which parallel the dietary energy contents and the resulting constant ($P>0.05$) caloric intake of hens receiving the various diets. A notable exception is the low caloric intake of hens fed the diet containing 15% dehulled fababeans. No logical explanation is available for this discrepancy. The similar ($P>0.05$) weight gain data agree with the energy intake data. Feed efficiency was the same ($P>0.05$) for all treatment groups except that hens fed 30% whole fababeans required more ($P<0.05$) feed to produce a unit egg mass than the other groups. This could be attributed to the high feed intake coupled with the reduction in egg weight effect associated with this diet. Mortality was low and was not related to any specific dietary treatment.

In the present study, laying hens adjusted their feed and therefore energy intake to compensate for the

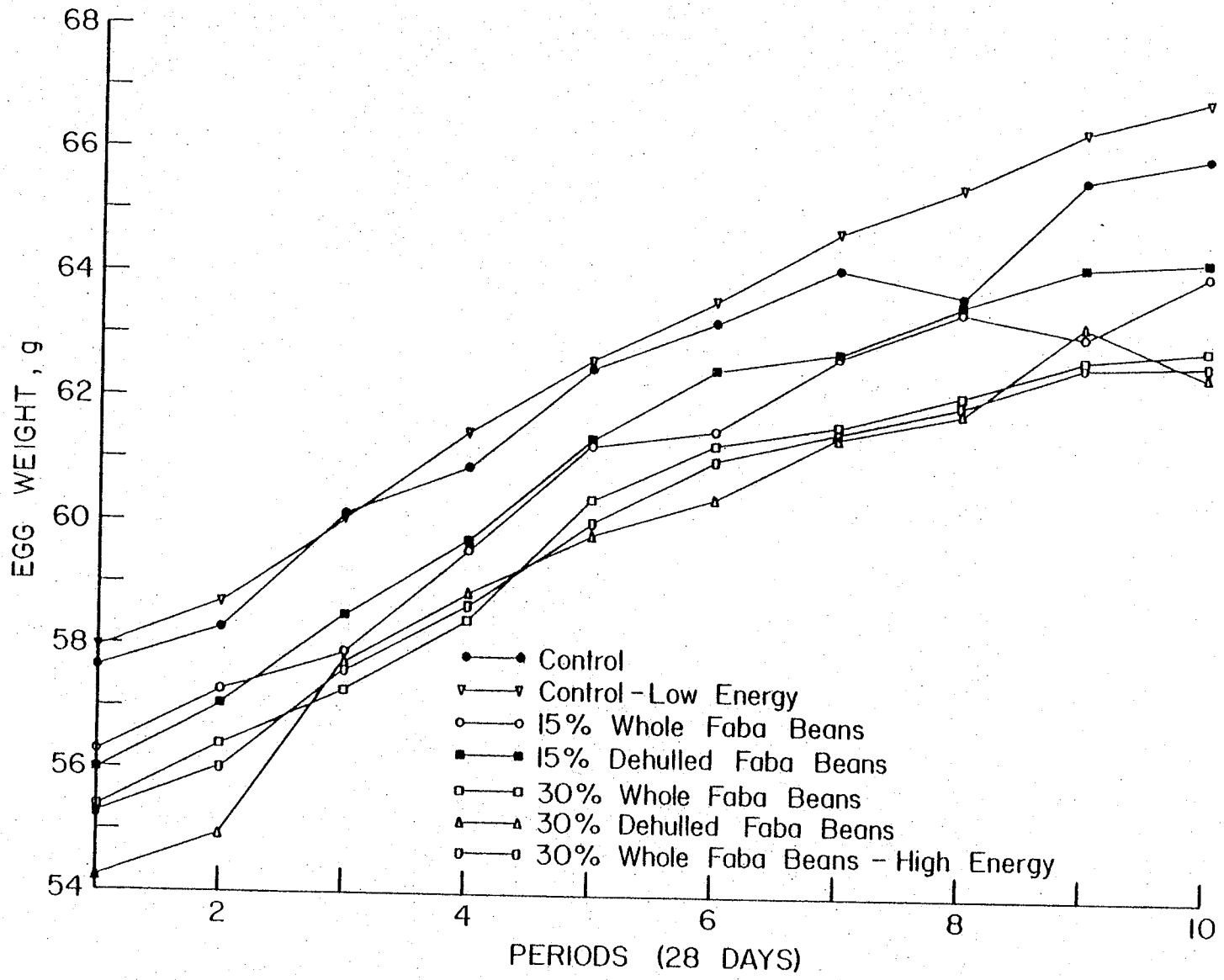


Fig. 6. Influence of whole and dehulled fababeans fed over a 280-day production period on egg weight (Experiment II).

apparent low metabolizable energy (M.E.) content of fababeans. It was postulated earlier (Campbell, unpublished data) that the tendency towards decreased egg production by hens receiving diets containing levels of fababeans in excess of 22 to 25% might be related to a depression in feed intake. The results of the present experiment, however, do not support this supposition. Furthermore, the calculated M.E. values in this study did not agree with the determined M.E. values (Table 3). This observation suggests that the variation might be related to the use in the formulation of laying hen diets of the M.E. values determined with growing chickens (Campbell, unpublished data).

The results of this experiment are in agreement with those of other workers in regard to egg weight in that egg weight was depressed with increased levels of fababeans in laying hen diets, but differ in regard to other parameters. Egg production was not affected by the level of fababeans in the diet. On the contrary, Davidson (1973), found that egg production was depressed with 15% fababeans. Feed efficiency was not affected with high energy diets containing as high as 30% fababeans. Vogt (1972), however, reported decreased feed efficiency with 20% fababeans. Weight gain which was similar for all groups in this experiment was reported to have been reduced in pullets fed 30% fababeans from 26 weeks of age (Lanza

et al. 1971). Robblee and coworkers (1977), reported that levels up to and including 20% fababeans in rations for laying hens had no adverse effect on mortality, egg production, feed efficiency or body weight. They, however, found that a level of 30% fababeans resulted in increased mortality, and decreased egg production and feed efficiency in one of two experiments conducted.

The consistency in egg weight depression in this experiment and the previous experiment is an indication that the egg weight depressing effect of fababeans might be detected over a relatively short period of time.

EXPERIMENT III

Influence of Dietary Methionine Levels

Supplementing a diet containing 25% fababeans with 0.05% DL-methionine supported as high ($P>0.05$) a rate of egg production as the control diet (Table 16). Fortifying such a diet with 0.14 or 0.23% DL-methionine did not have any beneficial effect on egg production ($P>0.05$). Egg weight of the group of hens fed the fababean diet supplemented with 0.05% DL-methionine was 5% ($P<0.01$) lower than that of the control. Additional supplementation (0.14 or 0.23%) of methionine to the fababean diet resulted in a significant ($P<0.01$) improvement in egg weight, however, the average egg weight was still slightly below that of the control group. A graphical presentation of egg weight

Table 16. Effect of Methionine Supplementation to Diets Containing 25% Fababeans on the Productive Performance of Laying Hens (Experiment III)

Treatments	Egg production (% hen day)	Egg weight (g)	Total egg mass (g/h/d)	Feed intake (g/h/d)	Feed efficiency	Weight gain (g)	Mortality %
Control	80.3±2.4[1]	57.7±0.6[B]	46.4±1.6	106.0±2.5	2.3±0.1	81.3±17.6	0.4
25% fababeans + 0.05% methionine	79.9±1.8	54.8±0.5[A]	43.8±1.0	105.6±1.7	2.4±0.0	150.7±21.6	0.8
25% fababeans + 0.14% methionine	80.9±2.8	56.2±0.4[B]	45.5±1.7	103.6±2.4	2.3±0.0	156.8±27.8	1.5
25% fababeans + 0.23% methionine	79.6±2.5	56.0±0.6[B]	44.5±1.2	103.3±1.9	2.3±0.1	161.0±24.5	1.1

[1]Means ±S.E. not followed by the same superscript letter within a column are significantly different at $P<0.01$.

results for hens fed the different diets also showed a consistent trend in which the control birds had the highest average egg weight throughout the 7, 28-day periods (Fig. 7). Total egg mass, feed intake and feed efficiency were not significantly ($P>0.05$) affected by dietary treatments. Birds fed diets containing fababeans tended to gain more weight, particularly as the level of methionine in the diet increased. This effect was, however, not statistically significant ($P>0.05$). Mortality was generally low although it tended to be higher with fababean diets as compared with the control diet.

In this study, the dietary levels of amino acids as determined from amino acid analyses are presented in Table 5. The percent dietary methionine and sulfur amino acids under these conditions were 0.35 and 0.63 in the control diet; 0.31 and 0.60 in the fababean diet supplemented with 0.05% DL-methionine; 0.48 and 0.76% in the fababean diet supplemented with 0.14% DL-methionine; and 0.58 and 0.92% in the fababean diet supplemented with 0.23% DL-methionine, respectively. The methionine intake, mg per hen per day, on the various diets was: 371, 327, 497 and 599 for the control and the three DL-methionine supplemented fababean diets, respectively.

The results of this experiment indicate that methionine levels above the NRC (1971 and 1977) requirements can significantly increase egg weight of laying hens fed fababean containing diets but that this effect is not pro-

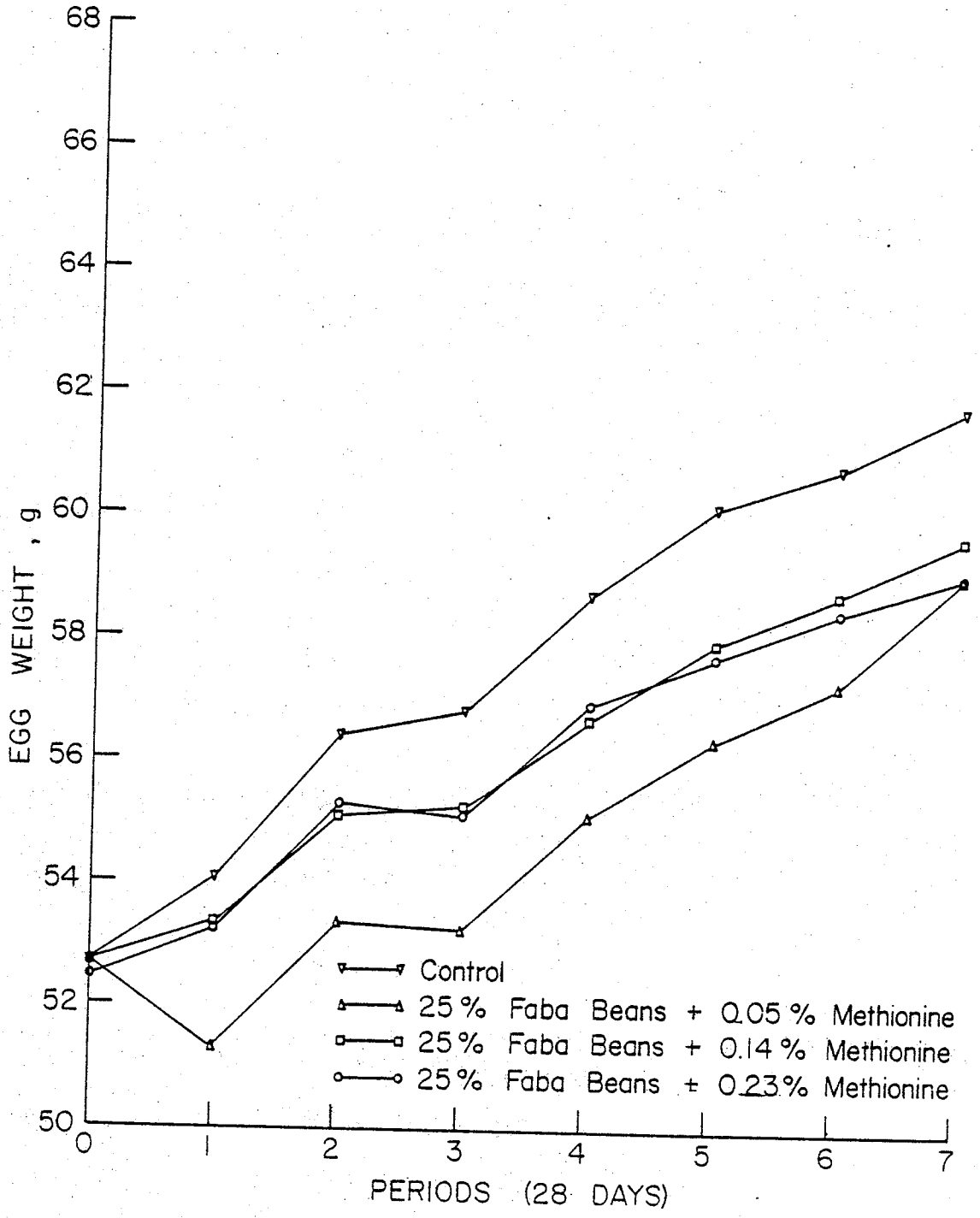


Fig. 7. Influence of dietary methionine levels on egg weight of laying hens fed diets containing 25% fababeans (Experiment III).

portional to methionine levels and that dietary methionine levels in excess of 0.48% have no additional beneficial effects with regard to egg weight. Other workers (Vogt, 1972; Davidson, 1973; Wilson and Teague, 1974; and Robblee et al., 1977), have reported that egg weight is depressed when fababeans are included in rations for laying hens and that the egg weight depression is only partially corrected by methionine supplementation to the diet. The apparent increased demand for methionine for egg weight but not for egg production was also observed by Jensen et al. (1974) using peas. The reason for the increased demand for methionine to maintain a high egg weight and body weight of hens fed diets containing fababeans is not clear. If the action of the egg weight depressing factor is similar to that of the growth inhibitors in raw soybeans, it is likely that fababeans cause an increased secretion of proteolytic enzymes into the intestinal tract of the hen which cause increased demand for methionine for the production of pancreatic enzymes. Methionine depletion due to its use as a methyl donor or for the synthesis of cysteine for detoxification of toxins present in fababeans could also increase the demand for methionine.

EGG WEIGHT DEPRESSING EFFECT OF FABABEANS WHEN FED TO LAYING HENS

EXPERIMENT IV

Influence of Dietary Energy and Protein Levels

Trial 1

Energy Level

The results of this study demonstrated that energy level significantly affected egg weight ($P<0.05$), feed consumption ($P<0.01$) and weight gain ($P<0.01$) and that protein source affected only egg weight ($P<0.01$) and feed consumption ($P<0.05$) (Table 17). Protein source had a more dramatic effect on egg weight but a much less marked influence on feed consumption or body weight gain than dietary energy level.

There was no interaction ($P>0.05$) between energy level and protein source for egg weight but there was a significant ($P<0.01$) interaction between these factors for feed intake and weight gain. It can be suggested from these results that the decrease in egg weight in fababean fed hens was independent of the energy level in the diet. Therefore, at any given energy level, diets that contained fababeans yielded eggs that were smaller than those obtained when hens were fed isocaloric diets in which soybean meal was the major source of dietary protein. The egg weight depressing effect of fababeans was evident within two weeks and persisted throughout the five-week experimental period (Fig. 8). The interaction observed for the other two performance criteria would suggest a different pattern

Table 17. Influence of Dietary Energy Level on the Egg Weight Depressing Effect of Fababeans (Experiment IV, Trial 1)

Energy level kcal M.E./kg	Fababeans			Soybean meal			Means for energy levels irrespective of protein source		
	Egg weight (g)	Feed consumption (g/h/d)	Body weight gain (g)	Egg weight (g)	Feed consumption (g/h/d)	Body weight gain (g)	Egg weight (g)	Feed consumption (g/h/d)	Body weight gain (g)
2430	58.3±0.4	110.3±2.1	23.0±14.0	59.9±0.5	118.4±1.7	19.8±12.7	59.1	114.4	21.4
2620	58.4±0.5	105.7±1.6	74.5±28.8	59.5±0.4	112.0±1.2	44.4±10.0	59.0	108.9	59.5
2800	59.5±0.4	113.1±2.2	110.5±13.8	60.9±0.4	109.3±1.6	73.5±10.3	60.2	111.2	92.0
2980	59.0±0.5	110.2±1.9	94.7±20.4	60.8±0.4	108.1±1.6	107.9±9.3	59.9	109.2	101.3
Mean	58.8	109.8	75.7	60.3	112.0	61.4			

Summary of Analysis of Variance

Source	df	Egg weight mean square	F	Feed consumption mean square	F	Weight gain mean square	
Energy level	3	10.15	3.59*	182.85	4.22**	36,687.43	10.05**
Protein source	1	61.88	21.87**	127.93	2.95*	5,712.00	1.56 NS
Energy level x protein source	3	0.81	0.29 NS	247.56	5.72**	34,783.43	9.52**
Error	104	2.83		43.31		3,652.20	

* P<0.05

** P<0.01

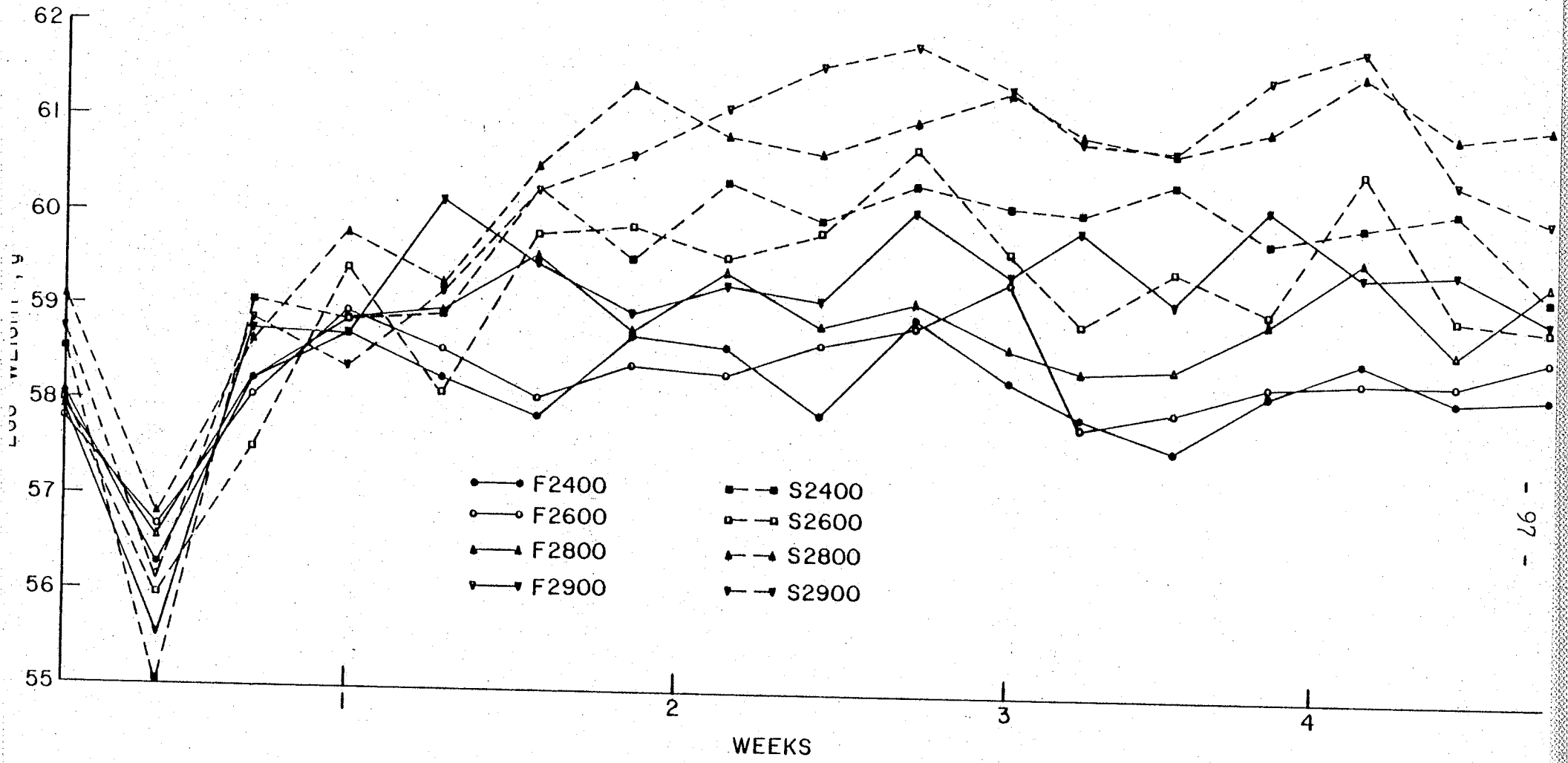


Fig. 8. The influence of dietary energy level on the egg weight depressing effect of fababeans (Experiment IV, Trial 1).

of response. Birds fed the diets that contained soybean meal reduced feed intake as the level of dietary energy was increased whereas similar compensatory decreases in feed intake did not occur with birds fed the fababean containing diets. No explanation for the body weight changes which were higher on the fababean diets than on the soybean diets is apparent.

The results of this trial would indicate that an energy deficiency *per se* is not a major factor in the egg weight depression resulting from feeding laying hens diets containing fababeans. A number of workers (Jensen *et al.* 1958; Menge *et al.* 1965 and Guenter *et al.* 1971), have reported that linoleic acid is essential for optimum egg weight. In the present study, linoleic acid deficiency does not appear to be involved in the egg weight depression caused by fababeans since fababeans are higher in linoleic acid (0.7%) than soybean meal (0.4%) (Blair 1977). Furthermore, soybean oil known to be high in linoleic acid was used to vary the energy densities of the diets.

The failure by laying hens fed fababean diets to adjust feed intake in the same manner as hens fed the soybean diets cannot be explained and is contradictory to the findings reported in Experiment II. The effect, however, appeared to be most pronounced for the low energy diets.

Trial 2

Protein Level

From the data presented in Table 18, it is evident that protein source affected both egg weight and feed consumption ($P<0.01$) while protein level affected only feed consumption ($P<0.01$). A protein level by source interaction for egg weight and feed consumption was not detected ($P>0.05$).

These results indicate that egg weight and feed consumption were lower with laying hens fed diets that contained fababean protein as compared to those fed soybean protein and that the difference occurred at all protein levels tested. It would appear, therefore, that the level of protein *per se* is not a major factor in the egg weight depressing effect of fababeans.

EXPERIMENT V

A Study of the Feasibility of Utilizing a 14-day Test Period

It was apparent from results obtained in previous experiments that the egg weight depressing effect of fababeans might be detected over a short period of time. This experiment was undertaken to study the feasibility of utilizing a 14-day test period to study the egg weight depressing effects of whole fababeans and fababean protein concentrate (FBPC).

The results of feeding diets containing 84% and 79% whole fababeans to laying hens over a 14-day period on

Table 18. Influence of Protein Level on the Egg Weight Depressing Effect of Fababeans (Experiment IV, Trial 2)

Protein level (%)	Fababean protein concentrate		Soybean meal		Means for protein levels irrespective of protein source	
	Egg weight (g)	Feed consumption (g/h/d)	Egg weight (g)	Feed consumption (g/h/d)	Egg weight (g)	Feed consumption (g/h/d)
14.2	61.7±0.4	98.6±1.9	62.1±0.6	103.7±1.1	61.9	101.2
15.7	61.4±0.5	97.3±1.9	62.6±0.4	102.5±1.8	62.0	99.9
17.2	61.0±0.4	90.9±1.5	62.1±0.4	104.7±1.3	61.6	97.8
18.7	61.5±0.5	102.6±1.0	61.8±0.5	109.6±1.7	61.7	106.1
Mean	61.4	97.4	62.2	105.1		

Summary of Analysis of Variance

Source	df	Egg weight mean square	F	Feed consumption mean square	F
Protein level	3	1.11	0.38 NS	208.14	6.25**
Protein source	1	15.92	5.43**	994.89	29.87**
Protein level x protein source	3	1.57	0.54 NS	8.85	0.27 NS
Error	104	2.93		33.31	

* $P<0.05$

** $P<0.01$

egg weight are presented in (Fig. 9). Although the birds were allotted to groups at random, egg weight of the treatment group was higher than that of the control group during the first control period. However, during the first test period, the egg weight of this group decreased and fell below that of the control group throughout the test period. In the second control period, the egg weight of the treatment group increased and was higher than that of the control group. It decreased and increased again during the second test and final control periods, respectively. A pattern in egg weight changes similar to that observed with whole fababeans was noted with the diet that contained fababean protein concentrate (FBPC) (Fig. 10). A decrease in egg weight of the treatment group occurred during the test period which was followed by an increase during the control period.

These results suggest that the 14-day test period could be utilized to detect the egg weight depressing effect of fababeans and fababean fractions, and that the egg weight depression appeared to occur within 3 to 4 days of feeding the treatment diets. It also required at least 4 days of re-feeding the control diet before the hens returned to the production of eggs of normal weight.

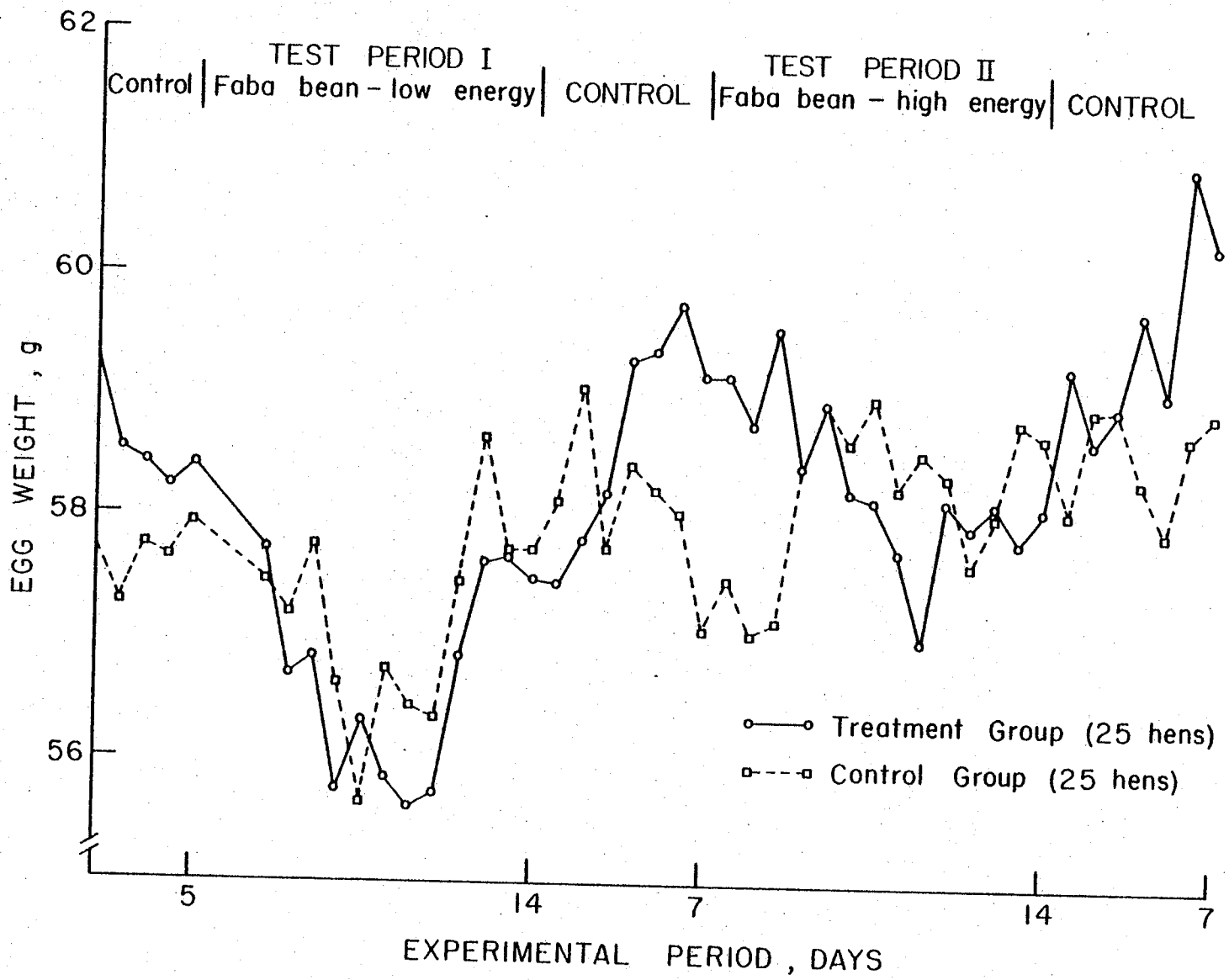


Fig. 9. Effect of feeding fababeans as the major component (79-84%) of low and high energy diets over a 14-day period on egg weight (Experiment V).

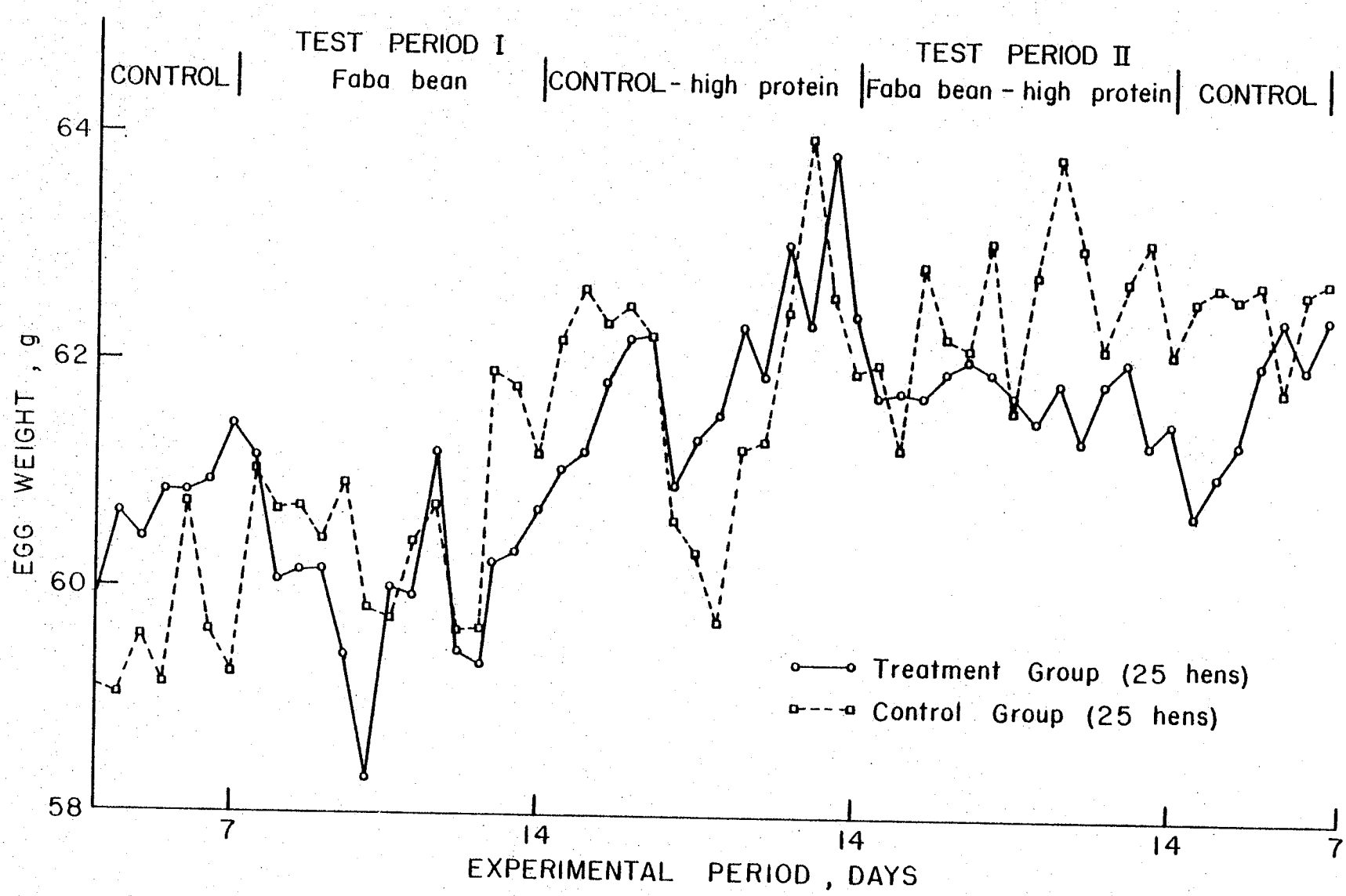


Fig. 10. Effect of feeding fababean protein concentrate as the major protein source of low and high protein diets over a 14-day period on egg weight (Experiment V).

THE USE OF SHORT DURATION TEST PERIODS TO STUDY THE EGG WEIGHT DEPRESSING EFFECT OF FABABEAN FRACTIONS AND TO ISOLATE THE EGG WEIGHT DEPRESSING FACTOR IN FABABEANS

EXPERIMENT VI

Influence of Autoclaving Treatment on the Egg Weight Depressing Effect of Fababean Protein Concentrate (FBPC)

The results of autoclaving treatment on the egg weight depressing effect of FBPC are summarized in Table 19 and the daily egg weight changes are presented in Fig. 11. Both untreated fababean protein concentrate (UFBPC) and autoclaved fababean protein concentrate (AFBPC) depressed egg weight significantly ($P<0.01$) as compared to the control. UFBPC, however, depressed egg weight to a greater extent than did the AFBPC. The patterns of egg weight depression and return to normal were similar in both tests. Egg weight depression commenced after 3 to 4 days of feeding the treatment diet and the time required for egg weight to return to normal levels depended on the extent of the depression. Feed intake appeared to be lower with UFBPC but not with AFBPC.

The results of egg component analyses are presented in Table 20. Wet yolk weight of eggs from the control group was 4.5% ($P<0.05$) greater than that of the treatment group which consisted of hens fed AFBPC. Other egg components were reduced proportionately. Yolk lipid content of eggs from the control group was 1.5% higher than that of eggs from the treatment group.

Table 19. Influence of Autoclaving Treatment on the Egg Weight Depressing Effect of Fababean Protein Concentrate (Experiment VI)

Period	Group[1]	Diet fed	Egg weight		Feed consumption
			Mean egg weight[2] (g)	Percent change[3]	Mean feed consumption (g/h/d)
Control I (14 days)	1	Control	54.7±0.5	---	100.7±1.6
	2	Control	54.6±0.6	---	101.9±1.6
Test I (14 days)	1	Control	56.3±0.5	102.9±0.5 A	106.9±1.7
	2	UFBPC[4]	53.4±0.5	97.8±0.6 B	97.9±1.3
Control II (14 days)	1	Control	57.5±0.6	---	99.2±2.3
	2	Control	56.7±0.5	---	101.1±1.6
Test II (14 days)	1	Control	58.5±0.6	101.7±0.4 A	102.9±2.2
	2	AFBPC[5]	56.3±0.6	99.3±0.5 B	102.2±1.9

[1]Number of hens in each group was 46.

[2]Mean ±S.E. were based on the average daily egg weight of 5 days per treatment during each period.

[3]Percent change in egg weight of each group during a test period in relation to a preceeding control period. Means for each test period not sharing a common superscript letter are significant at $P<0.001$.

[4]Untreated fababean protein concentrate.

[5]Autoclaved fababean protein concentrate.

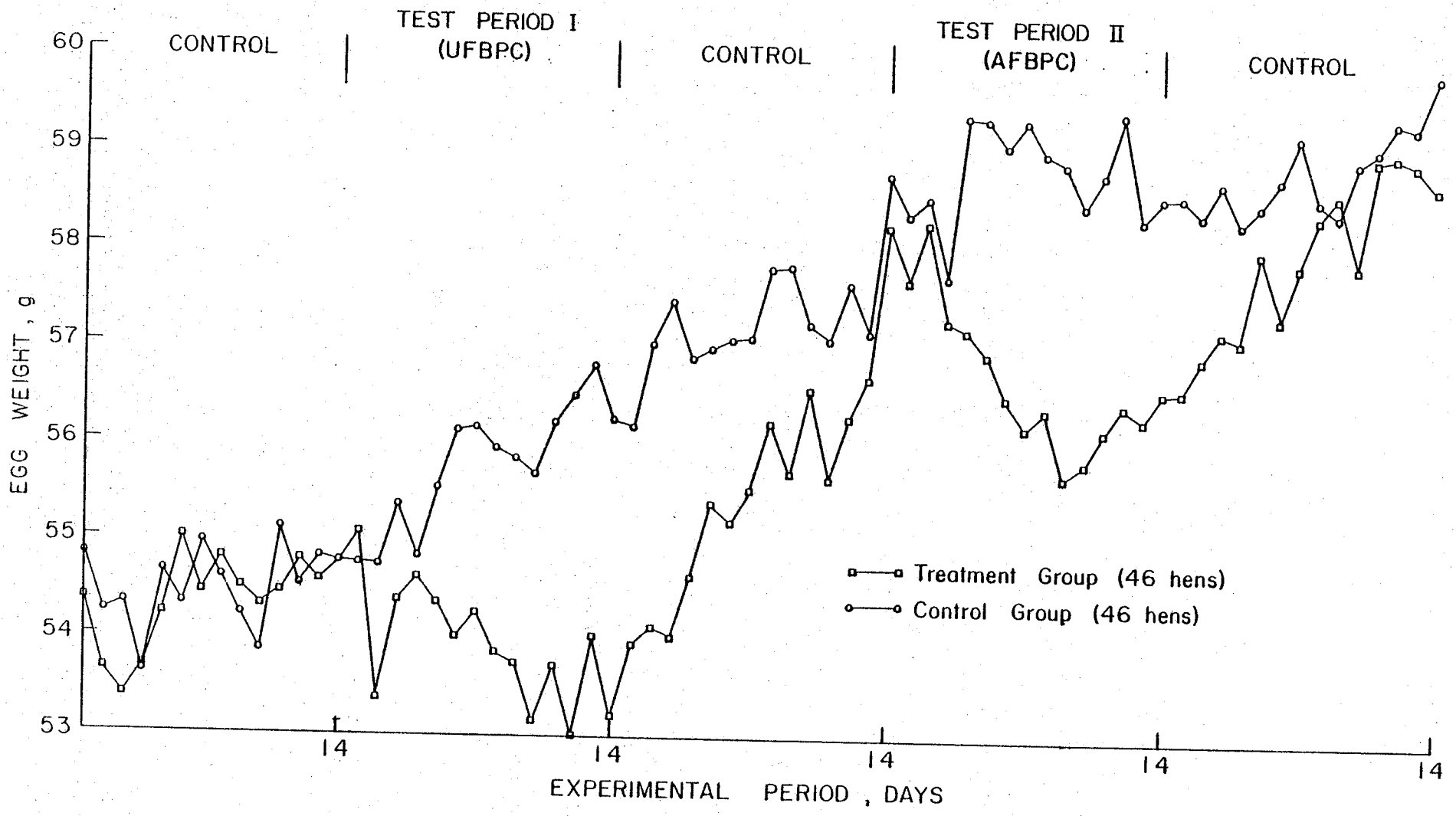


Fig. 11. Influence of autoclaving treatment on the egg weight depressing effect of fababean protein concentrate (Experiment VI).

Table 20. Influence of Feeding Autoclaved Fababean Protein Concentrate on Egg Components (Experiment VI)

	Control group	Test group
Number of eggs analysed	73	73
Egg weight (g)	59.9±0.6[1]	57.3±0.6
Wet yolk weight (g)	17.8±0.2[a]	17.0±0.2[b]
Dry yolk weight (g)	9.5±0.1	9.0±0.1
Dry yolk lipid (%)	65.8±0.3	64.8±0.1
Wet albumen weight (g)	32.9±0.4	31.5±0.4
Dry albumen weight (g)	4.5±0.1	4.3±0.1
Dry shell weight	5.4±0.1	5.1±0.1
Percentage wet yolk	29.7	29.7
Percentage wet albumen	54.9	54.9
Percentage shell	9.0	8.9

[1]Means ±S.E. not followed by the same superscript letter within a row are significantly different at $P<0.05$.

The results of this experiment indicate that the factor in fababeans that causes egg weight depression is associated with the protein fraction of the cotyledon and that the factor is relatively heat stable. These results confirm the results reported on the influence of heat treatment of fababeans on egg weight depression in Experiment I. It is suggested that the factor responsible for egg weight depression is not protein in nature since fababean proteins are rapidly denatured when subjected to 121°C for 30 minutes (Marquardt et al. 1975). Therefore, protein antinutritional factors such as trypsin inhibitors or hemagglutinins are probably not involved in egg weight depression. Marquardt et al. (1976), however, reported that vicine, a compound known to be present in fababeans and possibly associated with favism in humans, was not destroyed when fababeans were autoclaved at 121°C for 40 minutes or when the beans were extruded at 152°C. Olsen and Andersen (1978) found that pyrimidine glucosides, vicine and convicine seemed to be associated with protein bodies in raw fababeans and were absent from the hull. The highest content of these β-glucosides was found in a protein fraction produced by air-classification of dry milled fababeans (Olsen and Andersen 1978). The role of these glucosides, if any, in egg weight depression is not yet clear. It would appear, however, that egg weight depression is mediated via a reduction in the size of the

ovum ovulated which subsequently causes a reduction in the weight of other fractions of the egg.

EXPERIMENT VII

The Effect of Fababean Hulls on Egg Weight

It was shown earlier (Experiment I, trial 2) that fababean hulls supported the production of heavier eggs as compared to fababean cotyledons. The present experiment was conducted to establish the influence of the hull portion of fababeans on egg weight using the 14-day test procedure. The results are shown in Table 21 and the daily egg weight changes during the course of the experiment are presented in Fig. 12. The inclusion of 10% fababean hulls into a laying hen diet had no effect ($P>0.05$) on egg weight. Feed intake was similar for both the test and control groups.

The results of this experiment are in agreement with those reported in Experiment I, trial 2. It can be concluded, therefore, that the factor that causes egg weight depression in fababeans is not associated with the hull (testa) portion of the bean. It was also observed in Experiments I and VI, that this factor is relatively heat stable. The findings of Marquardt *et al.* (1977), that the condensed tannins of fababeans are heat labile and concentrated in the hull portion rule out the possibility of these compounds being involved in the egg weight depression. On the contrary, Tanguy *et al.* (1977), reported that con-

Table 21. Effect of Fababean Hulls on Egg Weight (Experiment VII)

Period	Group[1]	Diet fed	Egg weight: Mean egg weight[2] (g)	Egg weight: Percent change[3]	Feed consumption: Mean feed consumption (g/h/d)
Control (7 days)	1	Control	59.5±0.9	---	109.1±2.3
	2	Control	59.8±0.6	---	104.0±2.5
Test (14 days)	1	Control	60.1±0.8	101.0±0.6	106.8±2.7
	2	Hull	60.8±0.7	101.7±0.8	108.9±2.9

[1] Number of hens per group was 20.

[2] Means ±S.E. were based on the average daily egg weight of 5 days per treatment during each period.

[3] Percent change in egg weight of each group during the test period in relation to the control period. There are no significant differences in percent egg weight changes ($P>0.05$).

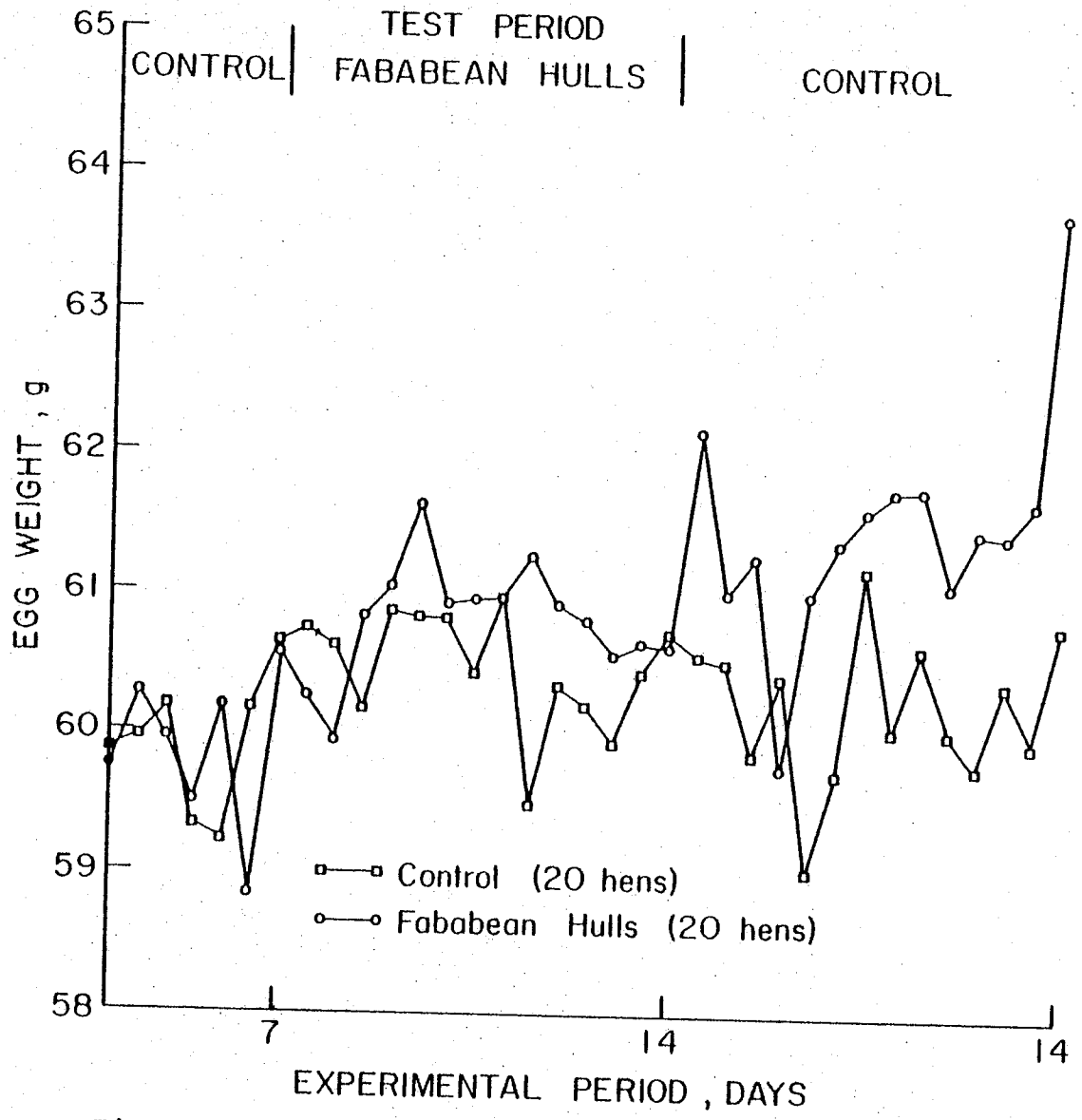


Fig. 12. The effect of fababean hulls on egg weight (Experiment VII).

densed tannins of horse bean seeds appeared to cause a reduction in egg weight of laying hens. The protein binding property of tannins was put forward as the explanation. If tannins reduced egg weight by reducing protein retention, the effect would probably have been more dramatic with the 10% hull diet used in the current experiment than with the diet used by Tanguy et al. (1977) which contained an equivalent of 4% hulls. Furthermore, the results reported in Experiment II, trial 2 demonstrated that protein level per se had no influence on the egg weight depressing effect of fababeans. It is apparent that a cause and effect relationship does not exist between fababean tannin content per se and the egg weight depressing effect, but rather this relationship exist between an anti-nutritional factor present in the cotyledon portion of the bean and egg weight depression.

EXPERIMENT VIII

The Influence of Fababean Starch and the Effect of Ethanol-Water Extraction of Fababean Protein Concentrate

Feeding a diet containing 26% fababean starch (FBS) to laying hens did not affect ($P>0.05$) egg weight (Table 22). In addition, no apparent effect on feed intake was evident. In contrast, addition of 2% ethanol-water extract prepared from autoclaved (121°C for 10 minutes) fababean protein concentrate (AFBPC) to a laying hen diet reduced egg weight significantly ($P<0.01$) as compared with the

Table 22. The Influence of Fababean Starch and the Effect of Ethanol-Water Extraction of Fababean Protein Concentrate on Egg Weight (Experiment VIII)

Period	Group[1]	Diet fed	Egg weight		Feed consumption
			Mean egg weight[2] (g)	Percent change[3]	Mean feed consumption (g/h/d)
Control I (7 days)	1	Control	59.0±0.6	---	102.8±2.9
	2	Control	59.1±0.6	---	107.0±2.9
	3	Control	59.6±0.8	---	103.7±4.1
Test I (14 days)	1	Control	61.3±0.6	103.9±0.5^{A}	104.0±2.2
	2	Starch	61.1±0.7	103.4±0.5^{A}	105.6±2.3
	3	Extract	59.0±0.7	99.0±0.9^{B}	98.9±3.2
Control II (14 days)	1	Control	61.3±0.6	---	104.5±1.5
	2	Control	62.1±0.6	---	101.7±2.1
	3	Control	61.5±0.7	---	98.8±2.9
Test II (14 days)	1	Control	61.6±0.6	100.5±0.5^{A}	102.1±1.7
	2	UFBPC[4]	59.5±0.6	96.8±0.5^{B}	94.6±2.0
	3	EFBPC[5]	60.5±0.7	98.4±0.5^{B}	95.0±2.9

[1] Number of hens in each group was 30.

[2] Means ±S.E. were based on the average daily egg weight of 8 days during each period.

[3] Percent change in egg weight of each group during a test period in relation to a preceeding control period. Means for each test period not sharing a common superscript letter are significant at $P<0.01$.

[4] Untreated fababean protein concentrate.

[5] Extracted fababean protein concentrate.

control diet. The egg weight depression occurred after 6 days of feeding the diet containing the extract (Fig. 13). The diet containing untreated fababean protein concentrate (UFBPC) or extracted fababean protein concentrate (EFBPC) significantly ($P<0.01$) depressed egg weight as compared to the control diet. Egg weight of the UFBPC diet was lower ($P<0.05$) than that of the EFBPC diet. Feed intake appeared to be depressed by the extract, UFBPC and EFBPC. Despite the lower feed intake, the methionine and lysine intakes, mg per hen per day, in the diets containing these fababean fractions were: 356 and 820; 341 and 653; 342 and 637, respectively.

These results suggest that fababean starch, prepared from the cotyledon portion of the bean contained little or no egg weight depressing activity. The results also confirm that FBPC prepared from the cotyledon portion of the bean causes egg weight depression and that the egg weight depressing factor in FBPC is extractable by ethanol and water although the procedure used was not completely effective in removing the active principle. Feed intake *per se* does not appear to be a major causative factor since birds consumed in excess of their daily recommended requirements for methionine and lysine as listed by NRC (1977).

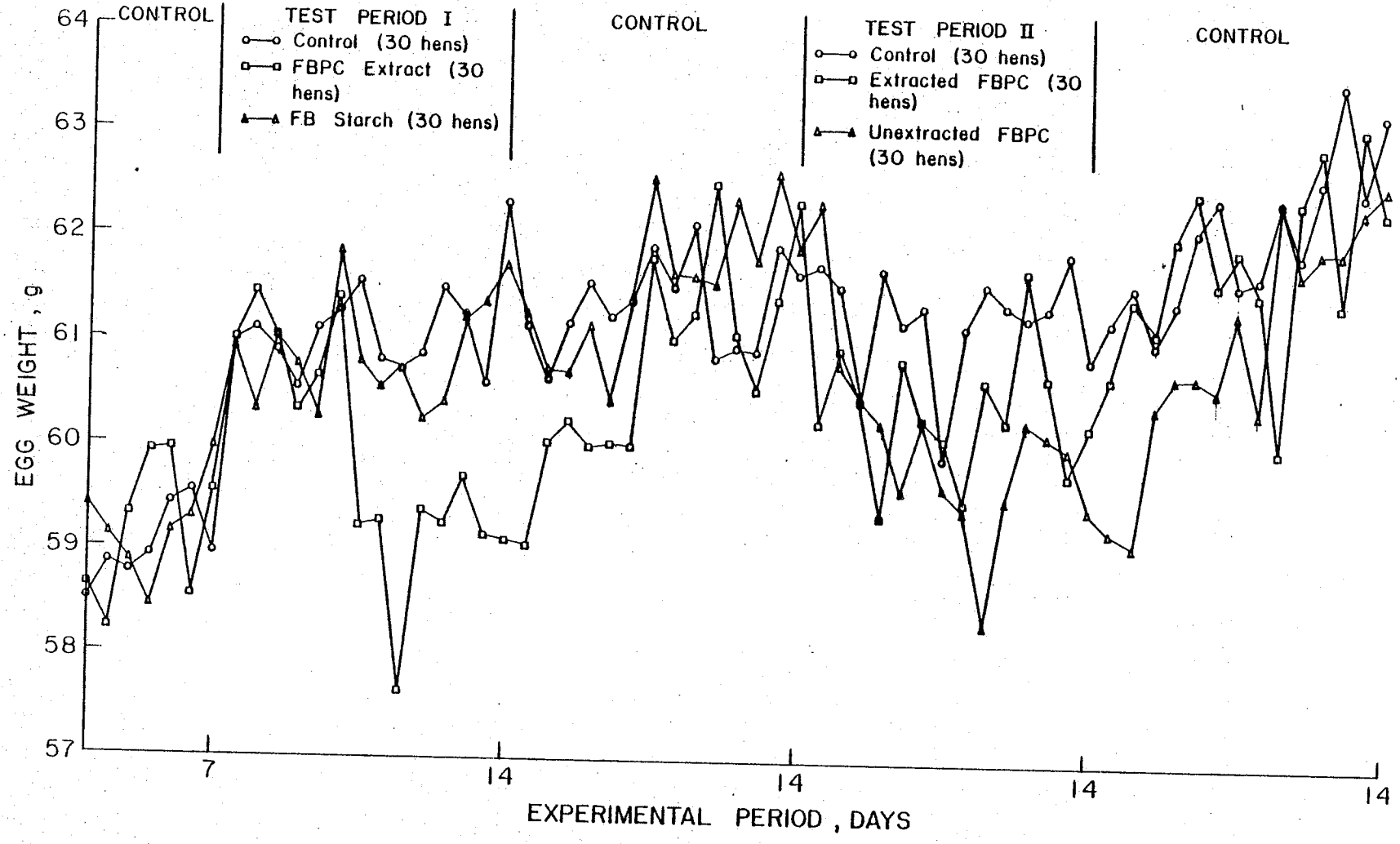


Fig. 13. The influence of fababean starch and the effect of ethanol-water extraction of fababean protein concentrate on egg weight (Experiment VIII).

EXPERIMENT IX

Further Studies with Ethanol-Water Extract of Fababean Protein Concentrate

The results of the influence of feeding different levels of ethanol-water extract of autoclaved fababean protein concentrate (AFBPC) over a 7-day period are shown in Table 23 and the daily egg weight changes during the course of the experiment are presented in Fig. 14. The diet containing 5% extract resulted in a rapid drop in egg weight which was significantly ($P<0.01$) greater than that of the control diet. In contrast, the rate and magnitude of decrease in egg weight was lower and not significant ($P>0.05$) with the diet that contained 2% extract. Feed intake was lower with the extract diets.

The results of this experiment confirm that an ethanol-water extract prepared from fababean protein concentrate (FBPC) when added to a laying hen diet depresses egg weight and that the magnitude and rate of depression in egg weight is influenced by dietary concentration of the egg weight depressing factor. The 7-day test period proved to be adequate for detecting the egg weight depressing effect of an ethanol-water extract of FBPC when fed at a high level.

Table 23. Further Studies on Egg Weight Depression with Ethanol-Water Extract of Fababean Protein Concentrate (Experiment IX)

Period	Group[1]	Diet fed	Egg weight		Feed consumption
			Mean egg weight[2]	Percent change[3]	Mean feed consumption
			(g)		(g/h/d)
Control (7 days)	1	Control	49.7±0.8	---	83.5±3.2
	2	Control	50.4±0.5	---	82.1±3.3
	3	Control	50.8±0.6	---	80.5±3.2
Test (7 days)	1	Control	51.6±1.0	103.8 ± 1.3^{A}	100.0±3.7
	2	2% Extract	51.1±0.7	101.4 ± 0.1^{A}	94.8±3.0
	3	5% Extract	48.7±0.6	95.9 ± 0.8^{B}	90.8±2.3

[1]Number of hens in each group was 30.

[2]Means ±S.E. were based on average daily egg weight of 6 days per treatment during each period.

[3]Percent change in egg weight of each group during the test period in relation to the control period. Means not sharing a common superscript letter are significant at $P<0.01$.

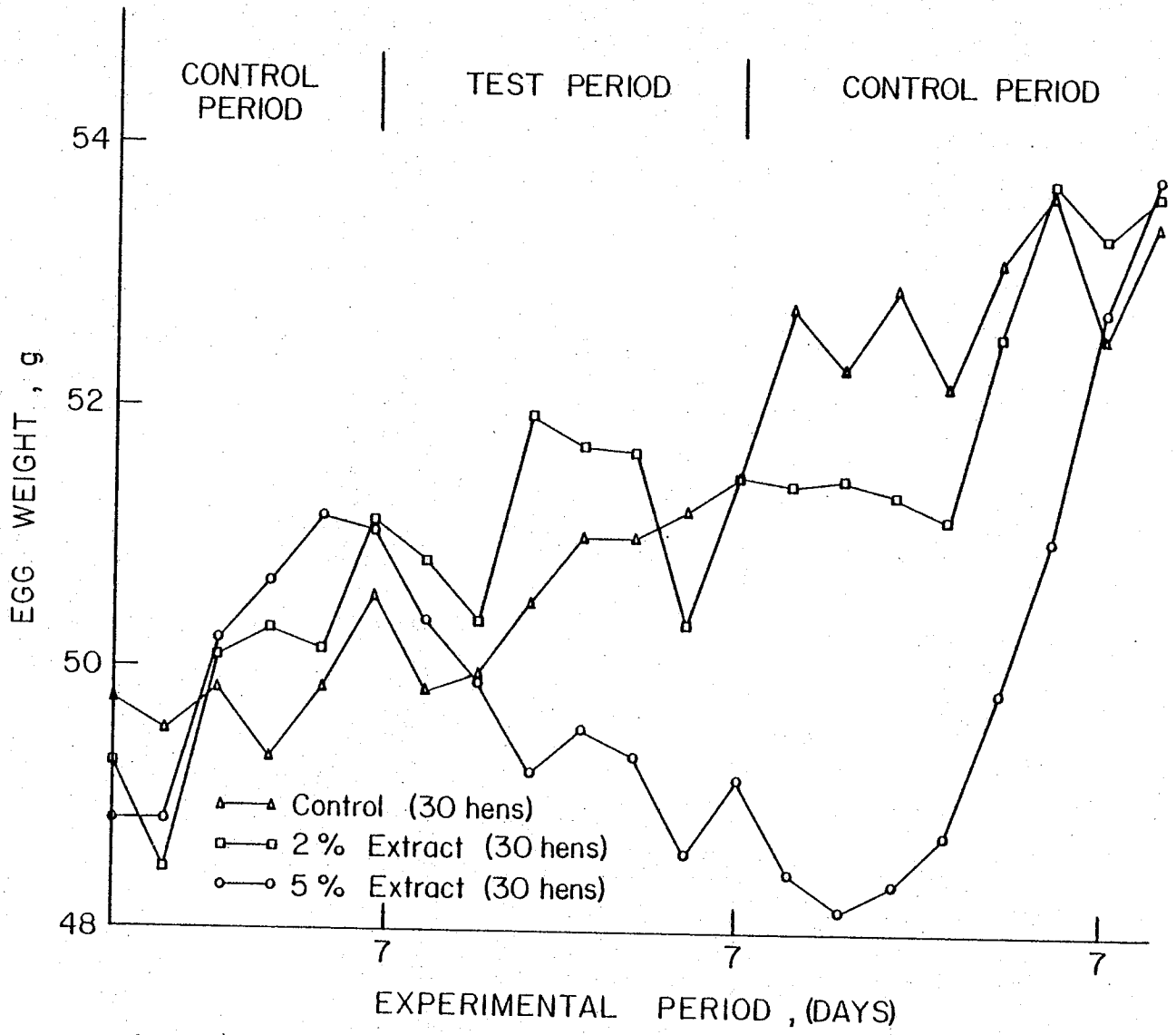


Fig. 14. Effect of feeding 2 and 5% ethanol-water extract of autoclaved fababean protein concentrate on egg weight (Experiment IX).

EXPERIMENT X

The Influence of pH Fractionation on an Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate

None of the fractions obtained by pH fractionation of the extract significantly ($P>0.05$) depressed egg weight (Table 24), although there was an apparent decrease in egg weight of birds fed the diet containing the soluble (supernatant) fraction (Fig. 15). Feed intake was slightly lower with the diets containing the pH fractions.

Since the ethanol-water extract had been shown to significantly depress egg weight (Experiments VIII and IX), the results of this experiment would suggest that pH fractionation of the extract might have destroyed the active component. The hydrochloric acid treatment of the extract followed by heat treatment of the supernatant in a cyclone evaporator, might have altered the active compound. This may be the case if the β-glucosides, vicine and convicine are involved in egg weight depression. Mager et al. (1969), reported the presence of β-glucosides, vicine and convicine in extracts from fababeans and found that these glucosides were easily converted by mild acid hydrolysis or by the enzyme β-glucosidase to their aglucones, divicine and isouramil. These aglucones are highly unstable in the presence of oxygen and are almost instantaneously destroyed by boiling.

Table 24. Influence of pH Fractionation on the Egg Weight Depressing Effect of Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate (Experiment X)

Period	Group[1]	Diet fed	Egg weight		Feed consumption
			Mean egg weight[2]	Percent change[3]	Mean feed consumption
			(g)		(g/h/d)
Control (7 days)	1	Control	57.2±0.5	---	78.7±3.4
	2	Control	57.3±0.9	---	85.2±3.2
	3	Control	57.6±0.8	---	80.0±3.0
Test (7 days)	1	Control	56.6±0.6	99.0±0.5	90.5±3.6
	2	Supernatant	56.2±0.7	98.1±0.4	87.5±3.4
	3	Precipitate	57.1±0.7	99.1±0.5	88.4±3.0

[1]Number of hens in each group was 30.

[2]Means ±S.E. were based on average daily egg weight of 5 days per treatment during each period.

[3]Percent change in egg weight of each group during the test period in relation to the control period. There are no significant differences in percent egg weight changes ($P>0.05$).

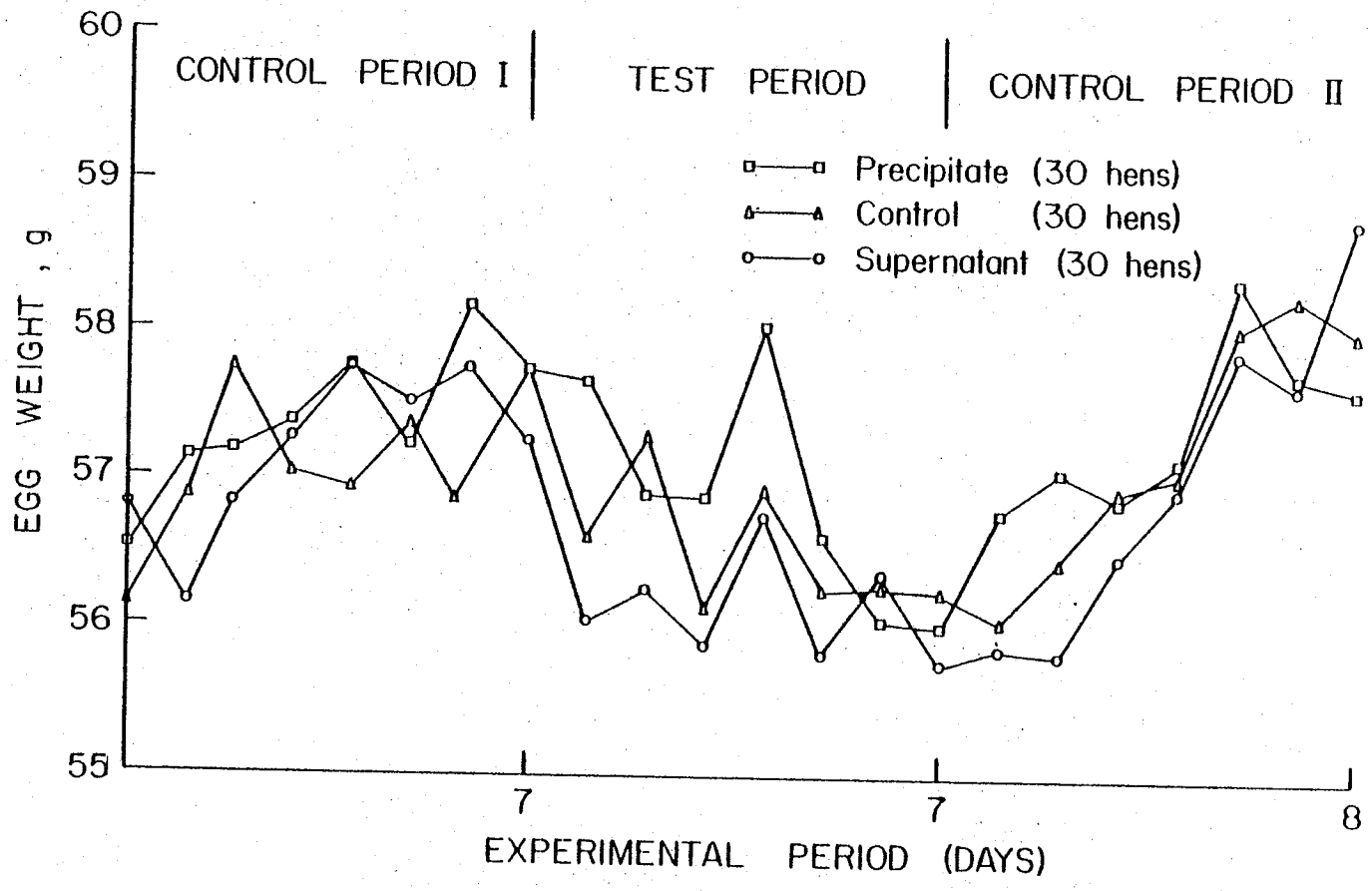


Fig. 15. The influence of pH fractionation of an ethanol-water extract of autoclaved fababean protein concentrate on egg weight (Experiment X).

EXPERIMENT XI

The Influence of Acetone Fractionation on an Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate

The results of feeding laying hens diets containing fractions obtained from water-acetone fractionation of an ethanol-water extract of AFBPC on egg weight and feed consumption are summarized in Table 25 and the average daily egg weight changes of each group are presented in Fig. 16. The group of birds fed the diet that contained ethanol-water extract had a significantly ($P<0.05$) lower egg weight than that of the groups of birds fed the other diets except for the group fed the diet that contained supernatant-H. The group fed the supernatant-H diet had a significantly ($P<0.05$) lower egg weight when compared to the control group but not ($P>0.05$) when compared to other treatment groups. There were no significant differences among egg weights of the groups fed the 1:1 water:acetone insoluble fraction, 1:3 water:acetone insoluble fraction, synthetic amino acid or control diets. Egg weight depression occurred after 3 days of feeding the treatment diets and returned to normal after 5 days of re-feeding the control diet. Feed intake was lower for groups fed the supernatant-H and extract diets. Liver weight of birds fed the control diet was 9% greater than that of birds fed the 1:1 water:acetone insoluble fraction, supernatant-H, amino acid or extract diets. The liver lipid content of the control birds was 33% greater than that of birds fed the supernatant-

Table 25. Influence of Acetone Fractionation on the Egg Weight Depressing Effect of an Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate (Experiment XI).

Period	Group[1]	Diet fed	Egg weight		Feed consumption
			Mean egg weight[2] (g)	Percent change[3]	Mean feed consumption (g/h/d)
Control (7 days)	1	Control	52.7±0.9	---	92.2±2.4
	2	Control	52.4±0.9	---	91.2±3.3
	3	Control	52.8±0.4	---	88.2±3.0
	4	Control	52.8±0.8	---	93.0±3.5
	5	Control	52.4±1.1	---	84.7±3.1
	6	Control	52.3±0.8	---	95.9±4.2
Test (7 days)	1	Control	52.8±1.2	100.2±1.0^{a}	98.2±3.3
	2	1:1 water-acetone fraction	51.0±1.2	97.3±0.9^{a,b}	100.2±4.2
	3	1:3 water-acetone fraction	51.8±0.3	98.1±0.7^{a,b}	100.0±4.4
	4	Supernatant-H	49.8±1.0	94.3±1.4^{b,c}	93.3±3.2
	5	Amino acids	51.4±0.8	98.1±1.5^{a,b}	95.5±4.3
	6	Extract	47.7±0.8	91.2±1.5^{c}	94.1±4.9

[1]Number of hens in each group was 20 (2 hens per cage).

[2]Means ±S.E. were based on average daily egg weight of 5 days per treatment during each period.

[3]Percent change in egg weight of each group during the test period in relation to the control period. Means not sharing a common superscript letter are significant at $P<0.05$.

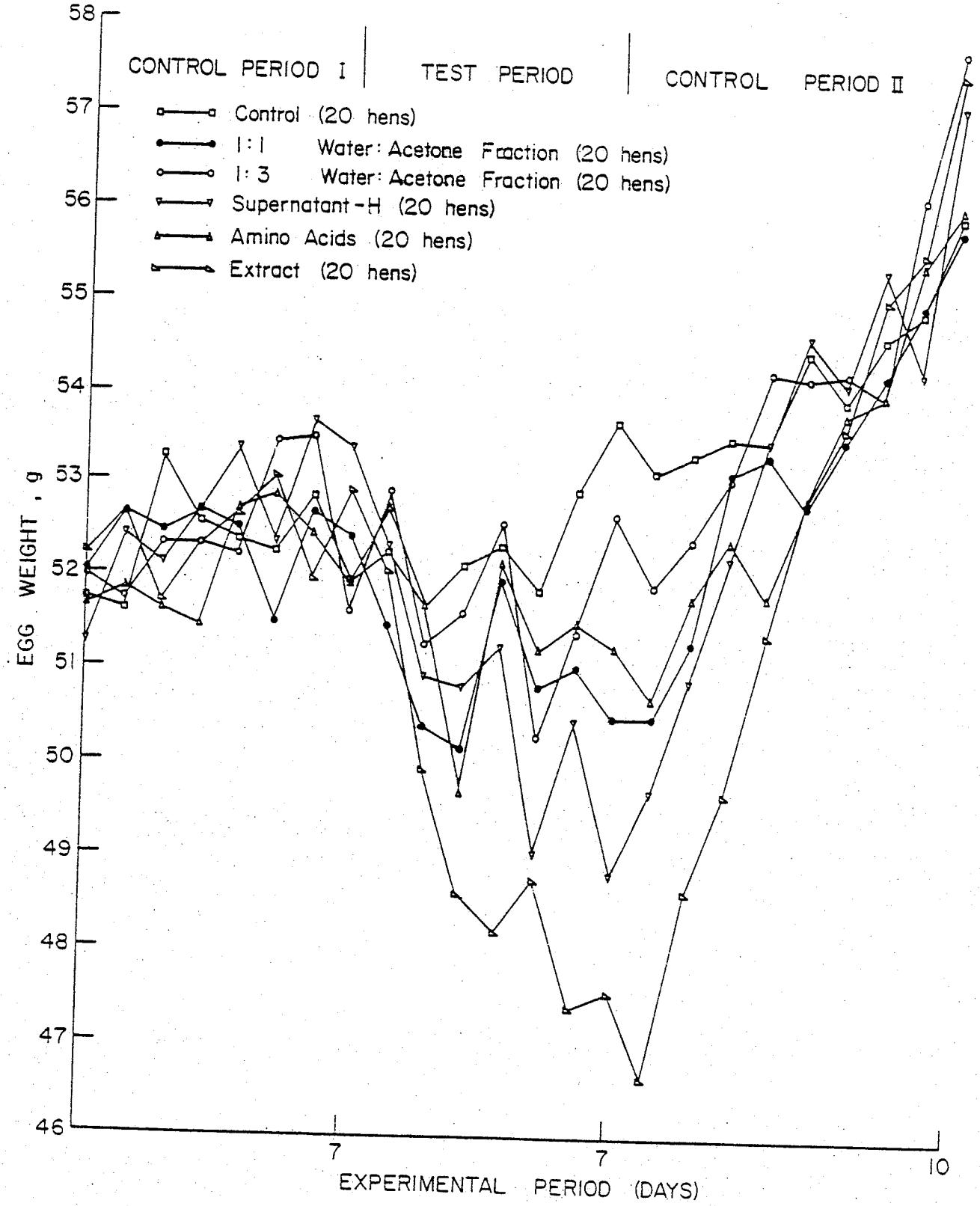


Fig. 16. The influence of water:acetone fractionation of an ethanol-water extract of autoclaved fababean protein concentrate on egg weight (Experiment XI).

H diet and 27% greater than that of birds fed the extract diet (Table 26).

The results of this study indicate that the fractions (extract and supernatant-H) that significantly depressed egg weight also reduced liver weight and liver lipid content. It was postulated from the results obtained in Experiment VI, that the egg weight depression is mediated via a reduction in the size of the ovum. Since it is generally accepted that yolk material is synthesized in the liver (Bell and Freeman 1971), it may be further postulated that the egg weight depressing factor causes a reduction in ovum size by altering the synthesis in the liver of yolk material.

The results of chemical analysis of the various fractions are presented in Table 27. The magnitude of egg weight depression seemed to be greater in those fractions (extract and supernatant-H) that contained the highest concentration of vicine reactive material. These fractions also had the highest levels of L-DOPA (0.3% and 0.5%, respectively). Supernatant-H which significantly depressed egg weight had the lowest total amino acid content. Amino acids that were present in either the extract or supernatant-H at levels greater than 1% were arginine, aspartic acid and glutamic acid. Since the addition of a mixture of synthetic amino acids to the control diet did not significantly affect egg weight, it may be postulated that the free amino acids including L-DOPA in the fababean extract

Table 26. Influence of Acetone Fractionation of an Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate on Weight and Lipid Content of Livers of Laying Hens (Experiment XI)

Diet	Liver weight (g/kg)	Liver lipid %
Control	30.3±2.0[1]	29.7±4.3
1:1 Water:acetone fraction	27.2±1.8	27.7±2.8
1:3 water:acetone fraction	29.2±1.5	22.2±3.1
Supernatant-H	27.5±1.9	19.7±1.5
Amino acids	27.4±2.0	28.2±2.5
Extract	27.7±1.3	21.7±3.3

[1]Means ±S.E. of 5 hens per treatment.

Table 27. Dry Matter, Protein, Vicine and Amino Acid Contents in Acetone Fractions of Ethanol-Water Extract of Autoclaved Fababean Protein Concentrate (Experiment XI)

	Ethanol-water extract	Water insoluble fraction (E)	1:1 Water: acetone insoluble fraction (C)	1:3 Water: acetone insoluble fraction (G)	Supernatant-H
	%	%	%	%	%
Yield	-	5.5	9.4	48.1	31.3
Dry matter	91.25	99.01	96.72	90.82	94.49
Protein (Nx6.25)	28.50	44.65	20.30	22.90	35.80
Vicine (OD^{650})[1]	0.295±0.029	0.055±0.029	0.019±0.007	0.184±0.055	0.455±0.051
Amino acids					
Lysine	0.67±0.04[2]	1.24±0.02	0.75±0.00	0.82±0.00	0.17±0.02
Histidine	0.27±0.01	0.51±0.01	0.28±0.00	0.30±0.00	0.11±0.01
Arginine	5.02±0.14	2.55±0.08	4.68±0.07	8.72±0.52	1.38±0.04
Aspartic acid	1.39±0.04	2.37±0.03	0.85±0.01	1.40±0.01	1.01±0.03
Threonine	0.35±0.02	0.76±0.01	0.25±0.00	0.39±0.00	0.19±0.01
Serine	0.38±0.01	0.95±0.01	0.28±0.01	0.34±0.00	0.25±0.00
Glutamic acid	2.00±0.10	3.43±0.14	1.35±0.04	2.18±0.03	1.14±0.06
Proline	0.42±0.00	0.90±0.02	0.18±0.01	0.35±0.00	0.35±0.04
Glycine	0.63±0.03	0.91±0.02	0.37±0.00	0.68±0.00	0.40±0.00
Alanine	0.47±0.02	0.88±0.04	0.22±0.00	0.49±0.00	0.28±0.01
Valine	0.33±0.01	1.08±0.05	0.13±0.00	0.27±0.00	0.22±0.02
Isoleucine	0.24±0.02	0.92±0.01	0.27±0.01	0.22±0.00	0.20±0.02
Leucine	0.35±0.02	1.65±0.01	0.27±0.03	0.32±0.00	0.28±0.03
Tyrosine	0.34±0.01	0.63±0.03	0.11±0.01	0.26±0.00	0.30±0.05
Phenylalanine	0.37±0.01	0.91±0.00	0.10±0.01	0.27±0.00	0.37±0.07
L-DOPA	0.26±0.00	0.04±0.01	0.05±0.01	0.09±0.01	0.52±0.01
TOTAL	13.49	19.73	10.14	17.10	7.17

[1] Vicine values as determined by the Higazi-Read (1974) method represent absorbancy units at 650 mu.

[2] Means ±S.E. based on duplicate samples.

are not the cause of egg weight depression.

Acetone fractionation proved to be a better method than pH fractionation for obtaining a fraction of an ethanol-water extract that significantly depressed egg weight.

EXPERIMENT XII

Isolation, Purification and Identification of the Egg Weight Depressing Factor

Fractionation of Supernatant-H

The results of the solubility characteristics of supernatant-H are presented in Table 28 and Fig. 17. Both methods of fractionation yielded the same amount of the white precipitate although the precipitate from the first method appeared to be more granular and lighter in color than the corresponding precipitate from the second method. The solubility of supernatant-H was diphasic (Fig. 17), indicating that there were two groups of components with different solubility characteristics. Extrapolation of the first part of the curve in Fig. 17 to the x axis, indicated that the first group of components would be completely (100%) dissolved when 1 g of supernatant-H was added to 8 ml of water. Extrapolation of the second part of the curve to the y axis showed that supernatant-H contained 13.60% (0.1360 g) of the white precipitate. When 1 g of supernatant-H was dissolved in water, however, only 87.43% (0.1189/0.1360) of the white material precipitated out

Table 28. Solubility of Supernatant-H in Water (Experiment XII)

Volume of water (ml/g sample)	Weight of the white precipitate (g)
2	0.2612±0.0053[1]
4	0.1701±0.0010
8	0.1189±0.0038
16	0.0848±0.0015
32	0.0339±0.0028

[1]Means ±S.E. based on duplicate samples.

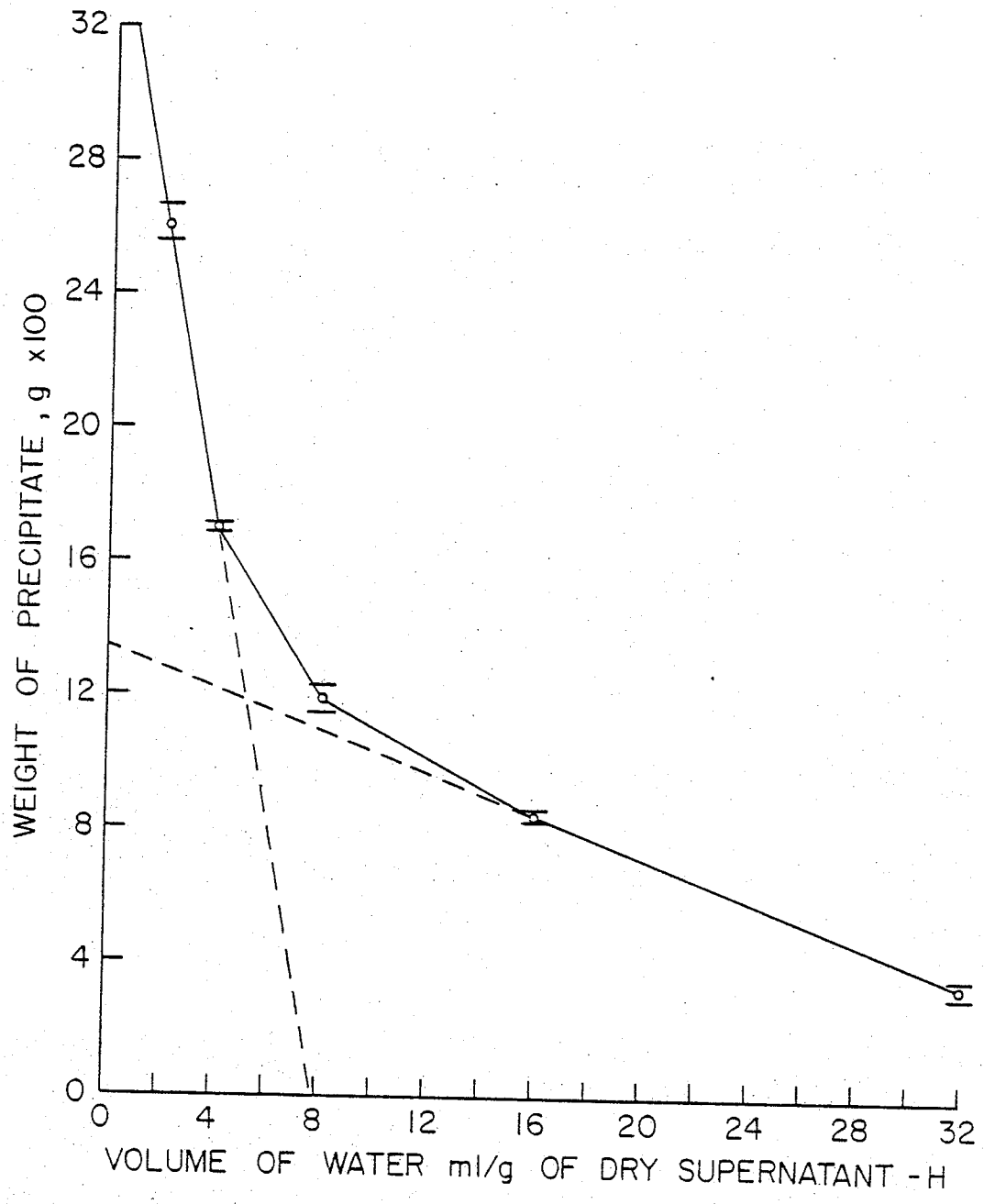


Fig. 17. Solubility of supernatant-H in water (Experiment XII).

(Fig. 17 and Table 28) and, therefore, 12.57% (0.1360-0.1189/0.1360) was in solution. Consequently the solubility of the white precipitate in water was calculated to be 0.0157 g per ml (0.1257/8) or 1.57 g per 100 ml. Lin and Ling (1962), reported vicine to be only slightly soluble in water (1 g in 100 ml) which agrees with the value obtained for the white precipitate.

Based on this solubility data a ratio of 1 g of supernatant-H to 8 ml (1:8) of water was adopted for the fractionation of supernatant-H into precipitate and soluble fractions for use in a laying hen feeding trial. A summary of the yield and chemical analyses of the white precipitate and the soluble fraction are presented in Table 29. A total of 1.35 kg of supernatant-H were fractionated to yield 11.45% of the white precipitate and 84.09% of the soluble fraction. The total recovery was 95.54%. The 1.35 kg of supernatant-H was equivalent to 4.31 kg of the ethanol-water extract or 40.10 kg of the AFBPC or 83.50 kg of dehulled fababeans. If whole fababeans (13% hulls) are used in the fractionation, the total amount required to provide the above yields would be 96.0 kg.

Analysis for nitrogen revealed that the white precipitate had a much higher nitrogen content (14.82%) relative to the soluble fraction (4.68%). An examination of the amino acid composition (Table 30) demonstrated that the amino acid contents of the white precipitate and the soluble fraction were 1.28 and 6.80%, respectively, which were much lower

Table 29. Chemical Analyses of the White Precipitate and Soluble Fractions of Supernatant-H (Experiment XII)

	Supernatant-H	White precipitate	Soluble fraction
Chemical analysis	%	%	%
Yield[2]	100.00±0.00[1]	11.45±0.00	84.09±0.00
Dry matter	93.63±0.15	93.53±0.03	94.08±0.01
Nitrogen	5.67±0.23	14.82±0.06	4.68±0.05
Vicine (OD^{650})[3]	0.033±0.003	0.111±0.015	0.027±0.004
Amount of vicine[4]	100.00±0.00	36.60±1.00	63.85±0.55

[1]Means ±S.E. based on duplicate samples.

[2]Amount of supernatant-H fractionated was 1.35 kg.

[3]Vicine values as determined by the Higazi-Read (1974) method represent absorbancy units at 650 mu.

[4]Amount of vicine in each fraction was calculated by multiplying the OD^{650} values per gram sample by the total weight of each sample.

Table 30. Amino Acid Composition of the White Precipitate and Soluble Fraction (Experiment XII)

	Soluble fraction	White precipitate
Amino acids[1]	%	%
Lysine	0.117±0.002[2]	0.027±0.004
Histidine	0.091±0.000	0.017±0.000
Arginine	1.402±0.026	0.140±0.008
Aspartic acid	0.982±0.009	0.207±0.009
Threonine	0.200±0.003	0.031±0.004
Serine	0.210±0.005	0.074±0.002
Glutamic acid	1.148±0.020	0.134±0.021
Proline	0.388±0.015	0.146±0.004
Glycine	0.383±0.007	0.140±0.003
Alanine	0.259±0.007	0.046±0.001
Valine	0.240±0.005	0.055±0.006
Isoleucine	0.201±0.001	0.080±0.009
Leucine	0.246±0.006	0.061±0.007
Tyrosine	0.243±0.009	0.067±0.002
Phenylalanine	0.340±0.010	0.056±0.004
L-DOPA	0.351±0.034	---
Total	6.801	1.281

[1]Ammonia, cystine and methionine were not included in the analyses.

[2]Means ±S.E. were based on duplicate samples.

than the protein contents when calculated according to the accepted formula of 6.25 x N. The high nitrogen content in these two fractions, particularly the white precipitate must, therefore, be associated with compounds other than proteins or amino acids. Bendich and Clements (1953) established the structure of vicine as 2, 6-diamino-4, 5-dihydroxy pyrimidine, 5-(β-D-glucopyranose) and that of convicine as 2, 4, 5-trihydroxy, 6-aminopyrimidine, 5 (β-D-glucopyranose). The contribution of these compounds to the nitrogen content of the fractions could account for the apparent discrepancy between amino acid and nitrogen contents. Furthermore, the results of vicine determinations indicated that the concentration of vicine increased by 236% in the white precipitate relative to supernatant-H and decreased by 18% in the soluble fraction. The total amount of vicine in the soluble fraction, however, was 75% more than in the white precipitate (Table 29).

Results of the feeding trial indicate that amino acids or L-DOPA, did not ($P>0.05$) depress egg weight (Table 31). In contrast, the white precipitate and the soluble fraction (supernatant) significantly ($P<0.01$) depressed egg weight. The pattern of egg weight depression and return to normal was typical of that caused by FBPC, ethanol-water extracts of FBPC and supernatant-H (Fig. 18). Feed intake was normal.

The results of this experiment together with those of Experiment XI confirm that amino acids including L-DOPA

Table 31. Influence of the White Precipitate and Soluble Fractions of Supernatant-H, and L-DOPA on Egg Weight (Experiment XII)

Period	Group[1]	Diet fed	Egg weight: Mean egg weight[2] (g)	Egg weight: Percent change[3]	Feed consumption: Mean feed consumption (g/h/d)[4]
Control (7 days)	1	Control	57.2±0.9	---	107.3±1.8
	2	Control	57.2±0.8	---	103.5±2.4
	3	Control	57.1±0.5	---	108.0±1.5
	4	Control	56.7±1.1	---	100.9±4.4
	5	Control	56.7±1.2	---	102.1±4.2
Test (7 days)	1	Amino acids	58.4±0.8	102.1±0.6 A	115.4±2.2
	2	White precipitate	54.6±0.8	95.5±1.2 B	109.3±2.0
	3	L-DOPA	58.7±0.7	102.8±0.6 A	110.2±1.9
	4	Control	56.7±0.5	100.0±1.7 A	114.4±3.6
	5	Soluble fraction	54.6±0.7	96.3±1.3 B	107.2±3.5

[1]Number of hens per group was 24.

[2]Means ±S.E. were based on the average daily egg weight of 5 days per treatment during each period.

[3]Percent change in egg weight of each group during the test period in relation to the control period. Means not sharing a common superscript letter are significant at $P<0.01$.

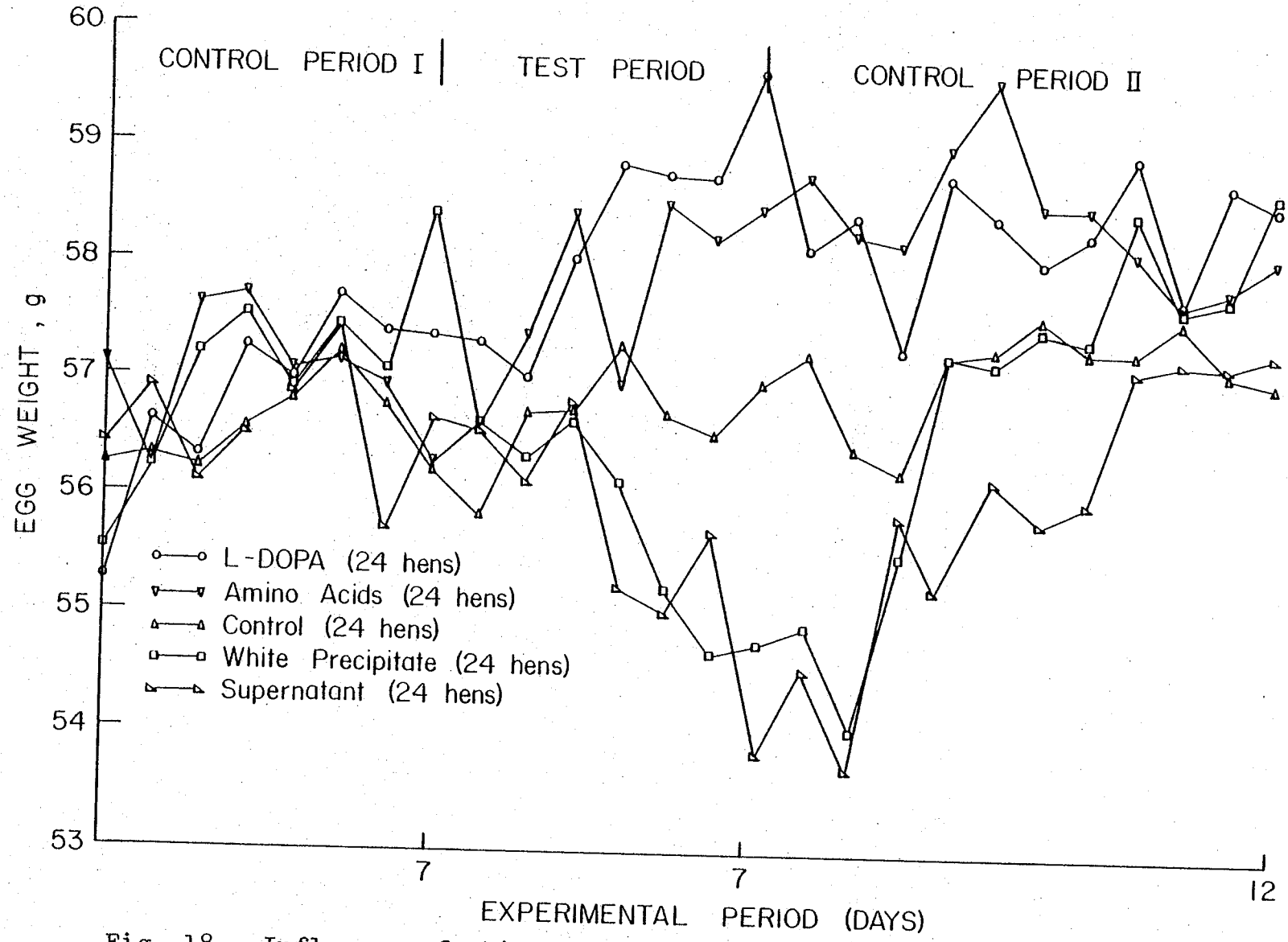


Fig. 18. Influence of white precipitate and soluble fractions of supernatant-H and L-DOPA on egg weight (Experiment XII).

are not the principle cause of egg weight depression but appear to implicate the aglucones, vicine and convicine as the major egg weight depressing factors. Additional information, however, is required in order to more accurately identify the egg weight depressing factor and to quantitate the levels of this factor in fababean fractions, particularly the white precipitate.

Purification of the White Precipitate

The solubility characteristics of the white precipitate at different temperatures are shown in Table 32. Solubility increased approximately 16 fold as the temperature was raised from 4°C to 80°C. The solubility characteristics in optical density units at different pH values are presented in Table 33. Starting from a pH of 6 for the saturated suspension of the white precipitate, and decreasing to a pH of between 2 and 3 increased absorbancy only 1.4 fold. On the other hand increasing the pH from 6 to between 9 and 10 increased absorbancy 3 fold. The yield of crystals of the egg weight depressing factor was increased from 21.5 to 46.5% by (1) washing with minimal volumes of cold water (2) maintaining a high pH and temperature for longer periods of time and (3) repeating each centrifugation step to collect the crystals that were decanted with the washing solution.

Table 32. Influence of Temperature on the Solubility of the White Precipitate (Experiment XII)

Temperature °C	OD^{280}
4	0.41±0.06[1]
20	1.80±0.02
40	2.30±0.06
80	6.63±0.46

[1]Means ±S.E. based on duplicate samples.

Table 33. Influence of pH on the Solubility of the White Precipitate (Experiment XII)

pH	OD^{280}
2.66±0.05[1]	0.70±0.01
4.08±0.09	0.66±0.02
6.28±0.05	0.50±0.02
7.74±0.09	0.53±0.01
9.37±0.02	1.58±0.05

[1]Means ±S.E. based on duplicate samples.

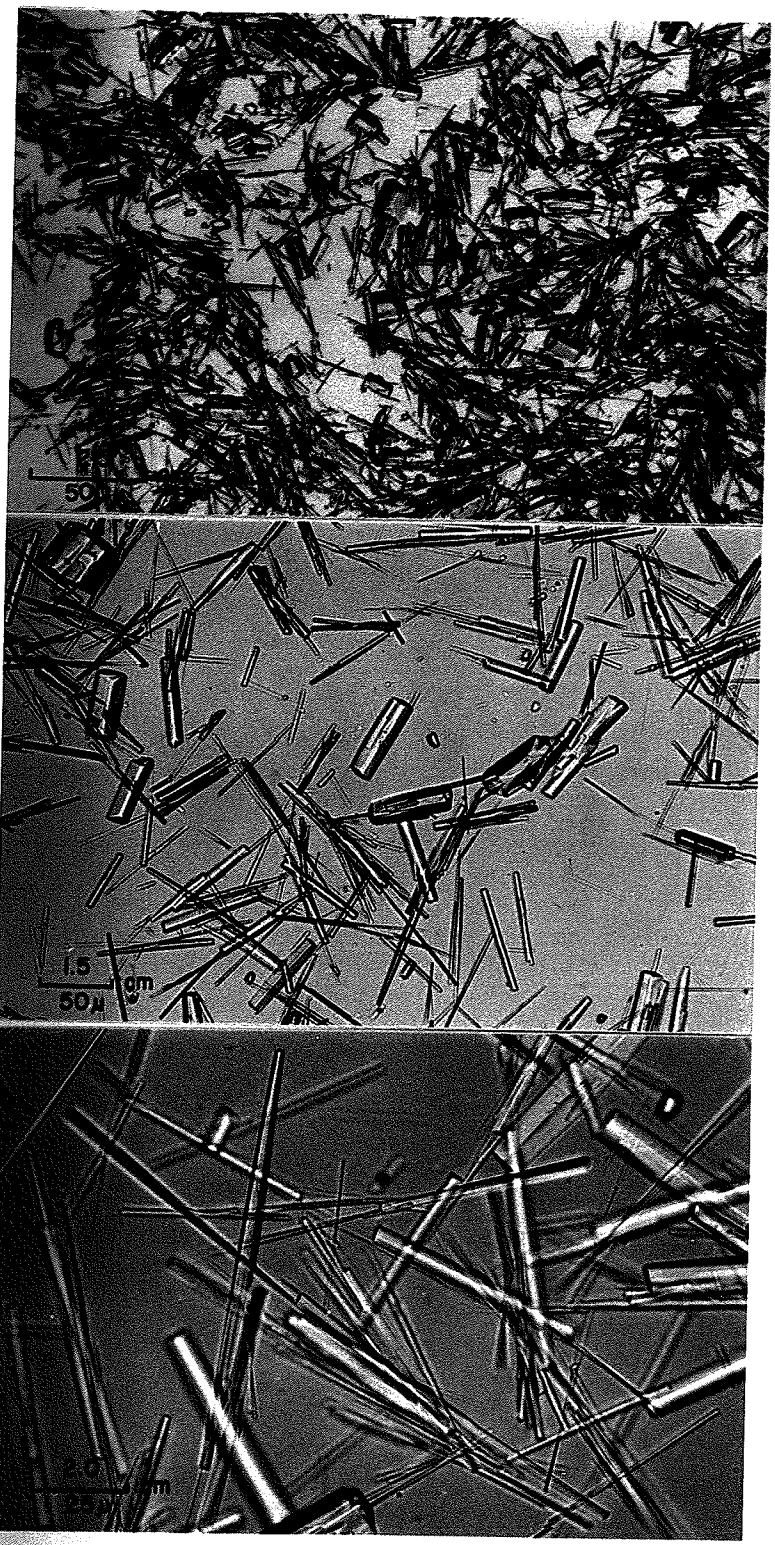
Identification of the Egg Weight Depressing Factor

In the previous study, the aglucones, vicine and convicine as components of the white precipitate were implicated in egg weight depression. Physical properties of crystals obtained from the white precipitate were studied to further verify that the major component was, in fact, vicine and/or convicine.

Pictures of the crystalline compound taken at different magnifications with a Zeiss photomicroscope are shown in Fig. 19. Two types of crystals were observed, colorless needle crystals and colorless prismatic crystals, the former crystals being more abundant than the latter. Lin and Ling (1962), reported that under the microscope, vicine showed uniform, colorless needle crystals and its aglycone, divicine, showed colorless prismatic crystals which stained brown when aged. A photograph taken under a Cambridge Stereo Scan Mark II is shown in Fig. 20.

The melting and decomposition values were 241-243, 242-244 and 242-244°C (uncorr.). These values are the same as the melting points of vicine listed by Bendich and Clements (1953).

The ultraviolet absorption spectra of the crystalline compound in acid, neutral and alkaline solutions are presented in Fig. 21. The calculated extinction coefficients of the compound are summarised in Table 34, assuming molecular weights to be 304 (vicine) or 322 (vicine plus one molecule of water) (Bendich and Clements 1953). The com-



(a)

(b)

(c)

Fig. 19. Vicine crystals in a water suspension. The pictures were taken with bright-field objectives on a Zeiss photomicroscope at 10-fold magnification (a); 63-fold magnification (b) and 160-fold magnification (c).

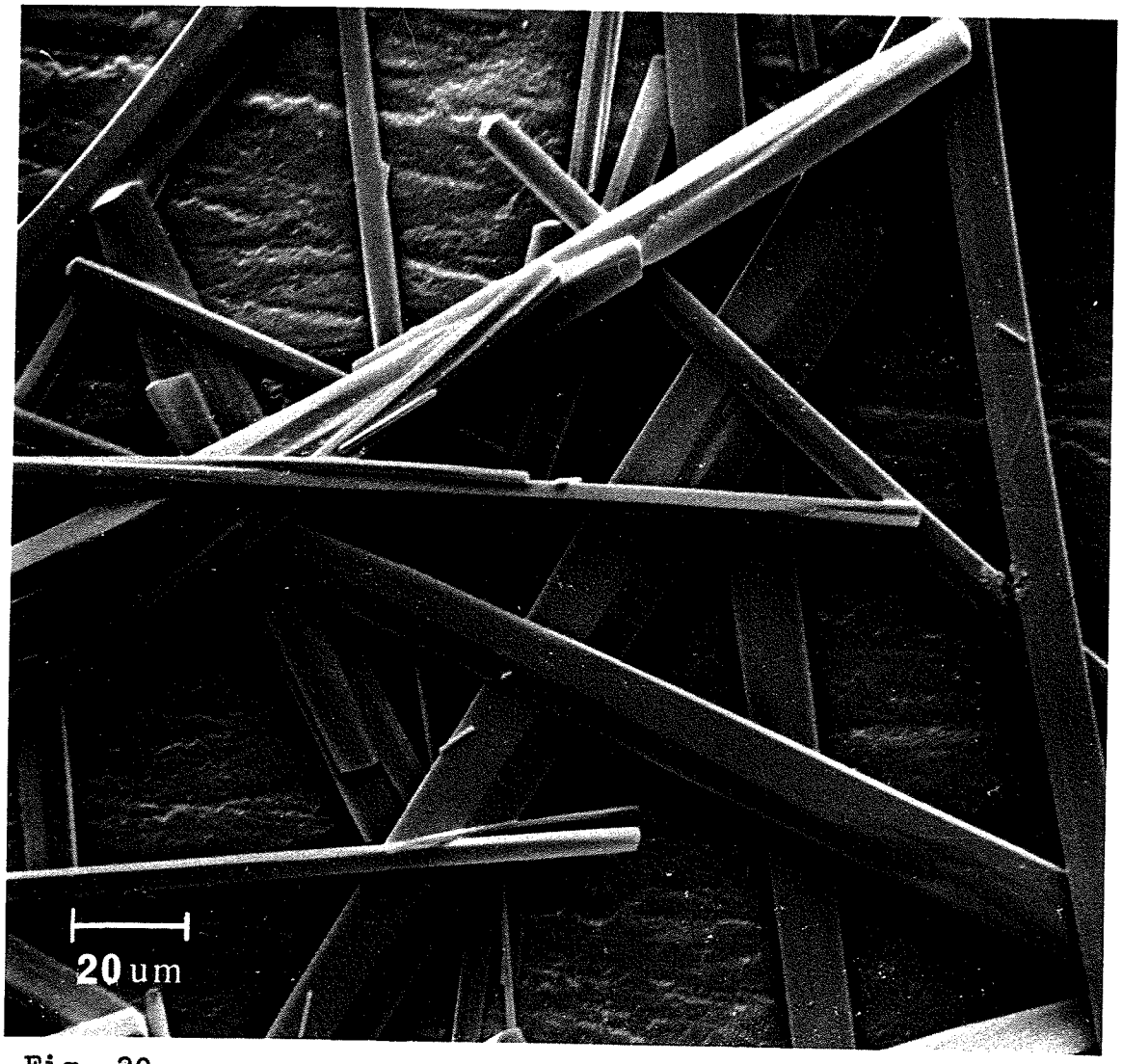


Fig. 20.
Vicine crystals. Picture taken with a Cambridge Stereo Scan Mark II at 750-fold magnification.

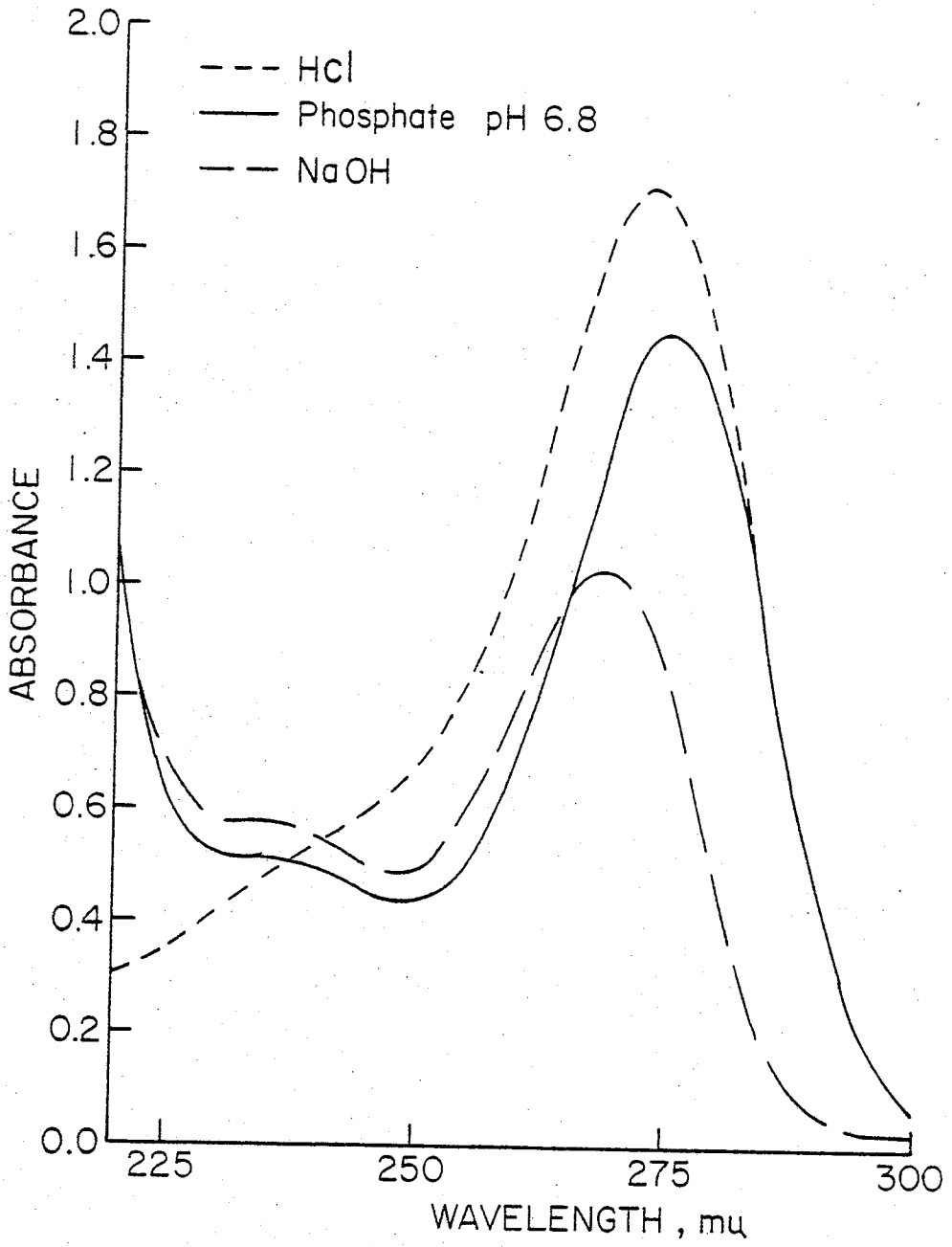


Fig. 21. Ultraviolet absorption spectra of the crystalline compound in acid, neutral and alkaline solutions (Experiment XII).

Table 34. Ultraviolet Absorption Spectra (Experiment XII)

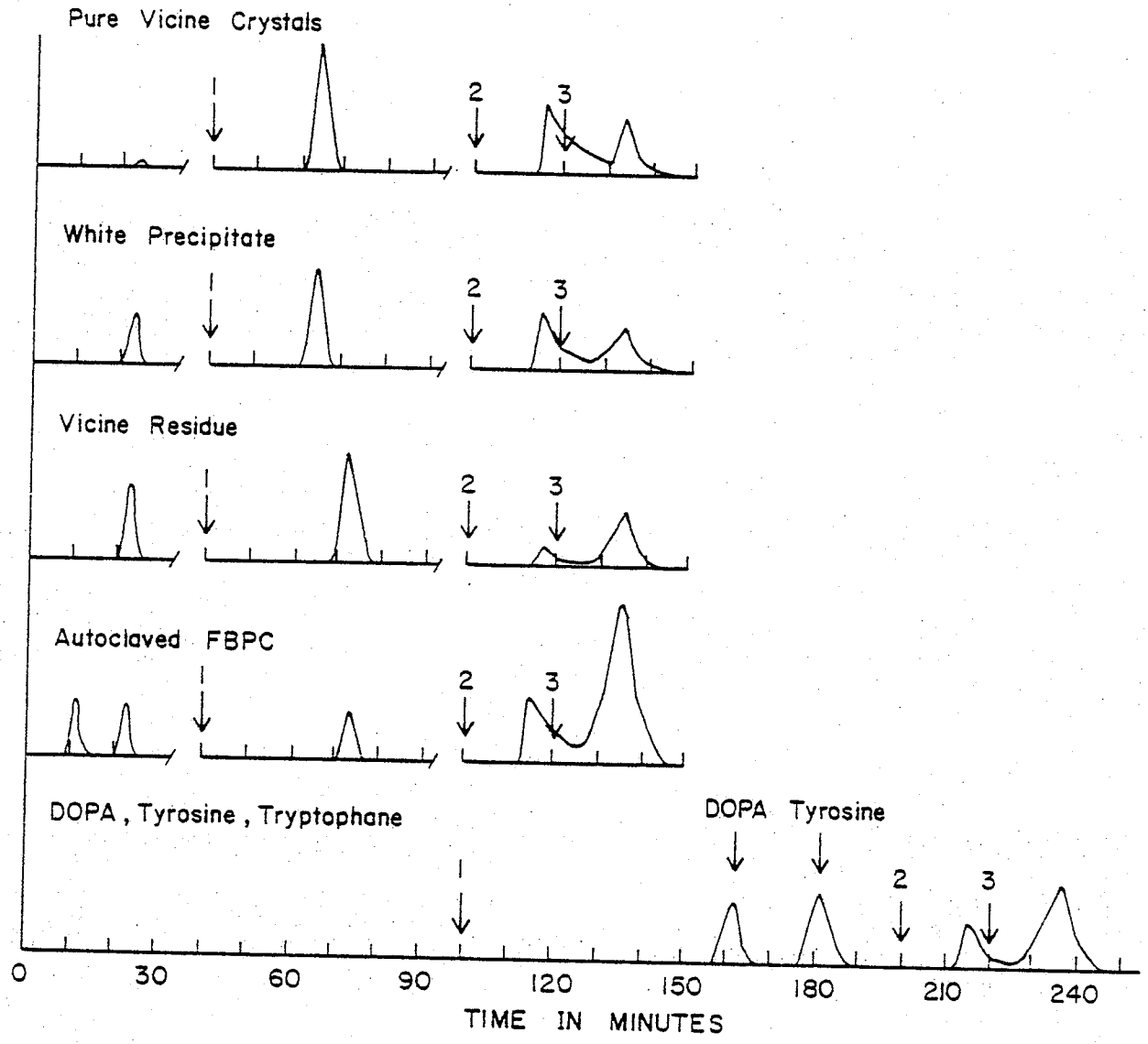
	Wave-length	E^1	
Medium	mu	304	322
0.1N HCl	274 max	15737.1±140.1[2]	16668.8±148.4
0.1N Phosphate pH 6.8	276 max	13448.9±0.0	14245.2±0.0
	250 min	3852.6±23.4	4080.7±24.8
	235 max	4506.4±23.4	4773.1±24.7
	231 min	4483.0±0.0	4748.4±0.0
0.1N NaOH	269 max	9993.3±747.1	10584.9±791.4
	248 min	4282.2±4.7	4535.7±4.9
	235 max	5136.7±46.7	5440.9±49.5
	230 min	5122.8±4.7	5426.1±5.0

[1]Molar absorption coefficient, calculated using the equation $E = \frac{A}{Cl}$ (Maron and Prutton 1958) where, A is the wavelength of light, C is the concentration of the solution and l is the cuvette length (1 cm), assuming a molecular weight of 304 for vicine and a molecular weight of 322 for vicine plus one molecule of water (Bendich and Clements 1953).

[2]Means ±S.E. based on duplicate samples.

pound showed maximum absorption spectra at 274 mu in acidic solution, 276 mu in neutral and 269 mu in alkaline solution and minimum at 250 mu and 248 mu in neutral and alkaline solutions, respectively. The ultraviolet absorption spectra of the crystalline compound are identical to those of vicine reported by Bendich and Clements (1953).

The elution profiles of the white precipitate, crystals, residue of crystallization and AFBPC are illustrated in Fig. 22. Studies with a blank sample that contained the diluting buffer yielded two peaks that corresponded with the last two peaks shown with each chromatogram. These results would suggest that the two peaks arose due to the interaction of the NaOH solution and the column eluting buffers and that they do not represent the absorbency of compounds applied to the column. The elution pattern of the crystals indicated a smaller peak which was eluted between 20 to 30 minutes after the sample was applied to the column and a major peak which was eluted between 20 to 40 minutes after the first buffer change. The white precipitate and the residue had patterns which were similar to that of the crystals except that the first peak was greater in the residue than in the crystals while the second peak was greater in the crystals than in the residue. The white precipitate had peaks which were intermediate between those of the crystals and the residue. Autoclaved fababean protein concentrate (AFBPC) yielded three peaks, two of which corresponded to those of the crystals, white precipitate and



1 End of First Citrate Buffer pH 3.25
2 End of Second Citrate Buffer pH 4.12
3 End of NaOH Wash

Fig. 22. Elution profiles of components of the crystalline material, white precipitate, residue, AFBPC and the ultraviolet absorbing amino acids (Experiment XII).

residue, while the additional peak was eluted prior to the first peak in the crystals, white precipitate and residue. The elution of concentrated preparations of the ultraviolet absorbing amino acids, L-DOPA, tyrosine and tryptophan yielded two peaks, the first being L-DOPA and the second, tyrosine. Tryptophan was not detected. None of these ultraviolet absorbing amino acids had elution profiles that corresponded with those observed with the fractions isolated from fababeans. The two peaks observed with the crystals, white precipitate, residue or AFBPC and the L-DOPA peak, correspond with the elution profiles of convicine, vicine and L-DOPA reported by Engel (1975).

The results of this study suggest that the white precipitate which contained only 1% amino acids was composed of two major compounds: one (convicine) appeared to be present in smaller amounts and could not be purified as it all remained in solution (residue of crystallization), and a second compound which was present in large amounts, reached a saturation point and formed crystals, which because of their physical properties were identified as vicine. The results indicate that the active egg weight depressing factor is either vicine or convicine or possibly a combination of the two.

The mode of action of the aglucones vicine and convicine in causing egg weight depression is not clear but may be similar to the etiology of favism in humans. The

consumption of fababeans has been associated with favism in humans. All attacks of the hemolytic anaemia have been limited geographically to families having low levels of glucose-6-phosphate dehydrogenase (G-6-PD) in their red blood cells. A number of workers (Mager et al. 1969) and Belsey, 1973) have demonstrated that haemolytic anaemia can be initiated in individuals having a low G-6-PD level by drugs or other compounds including the aglucones, vicine and convicine which oxidize reduced glutathione (GSH) to oxidized glutathione (GS-SG) in erythrocytes. Reduced glutathione (GSH) has a protective effect on the red cell membranes and the reduction of GS-SG to 2 GSH requires reduced nicotinamide adenine dinucleotide phosphate (NADPH) produced enzymatically by G-6-PD in the pentose phosphate pathway. NADPH produced in the pentose phosphate shunt is also used for the condensation of malonyl-CoA units during the synthesis of fatty acids. This effect may be related to the egg weight depression in laying hens fed fababean containing diets. Preliminary studies have indicated that egg weight depression is mediated via a decrease in yolk size which is primarily lipid and that the level of liver lipid was depressed. It may, therefore, be hypothesised that vicine and convicine reduce egg weight by producing divicine and isouramil which either oxidize NADPH to NADP or inhibit the synthesis and/or activity of certain dehydrogenases. The net effect is a reduction in the amount of NADPH available for the

synthesis of lipid for deposition in the ovum, which is reflected in egg weight depression. Since other components of the egg are added in proportion to the size of the yolk during egg formation a total reduction in egg size results.

Palatability does not appear to be the cause of reduced egg weight in hens fed fababeans, but rather a reduction in egg weight leads to a reduction in feed intake. This argument is supported by the finding that laying hens are able to consume more feed to compensate for the low nutrient content of the diet irrespective of whether fababeans are present in the diet or not. Furthermore, hens fed fababean diets gained more weight indicating the consumption of excess amounts of nutrients.

If the proposed mode of action of vicine and convicine is correct, then the etiology of favism in humans and egg weight depression in laying hens may have a common metabolic basis. Sturkie (1976), stated that birds may constitute more suitable models than rats for studying the carbon pathway of fatty acid synthesis and the regulation of lipogenesis in humans.

EXPERIMENT XIII

A Comparison of Chemical Analysis of Total Vicine in Various Fababean Fractions with the Observed Egg Weight Depressing Effect of these Fractions

The total vicine (vicine + convicine) or total ultraviolet absorbing material in various fababean fractions as estimated by the Higazi-Read and Collier methods or by a

column chromatographic method developed during this study is shown in Table 35. The results of the three assay methods were consistent. The hull and starch fractions, the fractions that did not depress egg weight (Experiments I, VII and VIII) had very little or zero total vicine content. These findings are in agreement with those recently reported by Olsen and Anderson (1978). The AFBPC had total vicine levels that were on average 25% lower than those present in the UFBPC. The egg weight depressing activity of the AFBPC was also shown to be less than that of UFBPC (Experiment VI). The concentration of total vicine reacting material increased with the potency of egg weight depression and the total amount of vicine (vicine + convicine) in the white precipitate was approximately 80 to 90%. The vicine content alone could only be estimated by the column chromatographic method which indicated that the white precipitate contained approximately 70% vicine while the crystalline material obtained from the white precipitate contained 96% vicine. It was concluded in Experiment XII, that the egg weight depressing factor is either vicine or convicine or possibly a combination of the two. Since the white precipitate which significantly depressed egg weight contained 70% vicine alone, it can be argued that vicine is the major egg weight depressing factor.

The results of chemical analysis indicate that egg weight depression bioassay can be effective in screening

Table 35. Quantitative Estimation of Vicine in Various Fababean Fractions (Experiment XIII)

Fababean fractions	Analysis method			
	Higazi-Read	Collier OD^{280}	Column chromatography	
	% Total vicine	% Total vicine	% vicine	% Total vicine
Hulls	0.02±0.00[1]	---	---	---
Starch	0.00±0.00	---	---	---
AFBPC[2]	0.94±0.07	1.08±0.09	0.43±0.03	0.85±0.08
UFBPC[3]	1.21±0.07	1.68±0.02	0.63±0.10	1.03±0.09
Extract of AFBPC	---	---	9.58±0.29	13.93±0.19
Extract of UFBPC	---	---	10.84±0.19	14.99±0.18
Supernatant-H	16.33±1.46	20.85±0.35	15.84±0.78	21.94±0.33
White precipitate	80.27±0.27	85.25±1.05	69.39±3.62	88.44±5.32
Residue	---	---	11.27±0.62	17.59±1.55
Crystals	100.00±0.00	100.00±0.00	96.00±0.35	100.00±0.00

[1]Means ±S.E. based on duplicate samples.

[2]Autoclaved fababean protein concentrate.

[3]Untreated fababean protein concentrate.

fababean fractions or varieties that contain vicine or vicine + convicine. The column chromatographic method developed in this study appears to be reliable since it gave total vicine values that were consistent with those of two established methods.

SUMMARY AND CONCLUSIONS

Studies were conducted to establish the effect of heat treatment, various levels of whole and dehulled fababeans and methionine supplementation on the productive performance of laying hens fed diets containing fababeans. Heat treatment (pelleting, extruding or autoclaving) of fababeans had no beneficial effect on the productive performance observed with laying hens. Laying hens adjusted their feed and, therefore, energy intake to compensate for the low M.E. content of diets that contained high levels of fababeans. Increasing the level of methionine in a diet containing 25% fababeans above that recommended by NRC (1971 and 1977) increased egg weight, but did not completely alleviate the egg weight depression. The increased methionine requirement for egg weight but not for egg production led to the postulation that methionine may have some role in the detoxification of the egg weight depressing factor.

Additional experiments were conducted to study the effect of dietary energy and protein levels on egg weight over a short time period (32 days). The results of these studies indicated that egg weight depression could be detected over even a shorter time period and that energy or protein level *per se* did not have a major effect on the egg weight depressing potential of fababeans. A 14-day test procedure which was later modified to a 7-day test procedure was, therefore, developed to study the egg weight

depressing effects of fababean fractions. Using this procedure, it was confirmed that the egg weight depressing factor is relatively heat stable, concentrated in the protein rich fraction of the cotyledon, and virtually absent from the hull and starch fractions of the bean. Trypsin inhibitors, hemagglutinins and tannins which are either heat labile or located in the hull fraction of the bean are, therefore, not involved in egg weight depression.

An ethanol-water extract of untreated fababean protein concentrate (UFBPC), significantly ($P<0.01$) depressed egg weight indicating that the egg weight depressing factor was extractable in an ethanol-water mixture. Further studies with the extract showed that the magnitude and rate of egg weight depression was dependent on the concentration of the causative principle. Attempts were made to fractionate an ethanol-water extract prepared from autoclaved fababean protein concentrate (AFBPC) in order to concentrate and possibly isolate the egg weight depressing factor. Fractionation using hydrochloric acid to lower pH produced two fractions none of which depressed ($P>0.05$) egg weight. Since the extract had been shown to depress egg weight, it was concluded that HCl treatment inactivated the active component. Acetone fractionation of the extract yielded four fractions, one of which (supernatant-H) significantly ($P<0.05$) depressed egg weight. Supernatant-H was further fractionated after studying its solubility characteristics

in water, into a white precipitate and a soluble fraction (supernatant). Both these fractions significantly ($P<0.01$) depressed egg weight. The egg weight depressing activity, however, was more concentrated in the white precipitate which represented only 11.43% of the original amount of supernatant-H fractionated. The solubility characteristics of the white precipitate at different temperatures and pH values were studied in order to purify this fraction by re-crystallization. Various physical properties of the crystals obtained were found to be identical to those of vicine. A second compound, convicine, was shown to be present mainly in the supernatant (residue) of crystallization. It was concluded, therefore, that the egg weight depressing factor is vicine or more likely a combination of vicine and convicine with vicine being the major principle.

Quantitative estimation of total vicine (vicine + convicine) in the various fababean fractions indicated that the fababean fractions (hulls and starch) that did not depress egg weight contained little or no vicine activity. On the contrary, the potency of egg weight depression increased with increases in the vicine content of the fractions. The mode of action of vicine and convicine in egg weight depression was postulated to have a similar chemical basis to etiology of favism in humans.

Conclusions

1. In general fababeans may be added to laying diets as the major source of protein without adversely affecting egg production. However, egg weight depression results. The egg weight depression can be considered an economic disadvantage to producers, but could be an advantage in certain situations where the production of medium size eggs with superior shell quality is required for most of the production cycle.
2. Heat treatment of fababeans does not alleviate the egg weight depressing effect of the beans.
3. Methionine supplementation to fababean containing diets at levels above NRC (1971) requirements significantly improves egg weight and tends to cause obesity in laying hens.
4. Laying hens are able to adjust their feed intake to compensate for the low energy content of the diet irrespective of whether fababeans are present in the diet or not.
5. Energy and/or protein level in the diet does not influence the egg weight response to fababean feeding.
6. The egg weight depressing effect of fababeans which can be detected within 3 to 4 days is associated with the protein rich fraction of the cotyledon but is absent in the hull or starch fractions of the bean.

7. Ethanol-water extracts of untreated or autoclaved (121^{o}C for 10 min) fababean protein concentrate depress egg weight.
8. Fractionation of the ethanol-water extracts using acetone, followed by crystallization out of the supernatant through re-crystallization with water leads to the production of crystalline vicine which is the major egg weight depressing factor in fababeans.

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