THE UNIVERSITY OF MANITOBA

EVALUATION OF LATHYRUS (*Lathyrus sativus* Linn. var. seminis albi) AS A FEEDSTUFF FOR POULTRY

by

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ABSTRACT

A series of experiments were conducted with growing chicks in an attempt to (1) observe the detrimental effects associated with the feeding of lathyrus, (2) develop dietary formulations and processing methods that would minimize these effects, and (3) establish a basis for future experiments. Various dietary treatments were administered in feeding trials designed to study the effects on chick performance of increasing dietary levels of lathyrus, long term (4 weeks) feeding effects of lathyrus, varying energy and protein densities, amino acid supplementations, autoclaving of lathyrus for increasing time periods, and different kinds of lyophilized extracts of lathyrus.

The results indicate that several factors may affect the utilization of lathyrus by growing chicks. Methionine was the first limiting amino acid in lathyrus. Proportional decreases in growth performance were observed in chicks fed increasing levels of lathyrus, and both energy and protein were less available in lathyrus than in a soy-wheat based diet. However, the results from the pair-feeding of soy-wheat to lathyrus showed that the poor performance with the lathyrus fed birds cannot be attributed solely to an appetite or palatability effect. Chicks seemed to adapt to the adverse effects of lathyrus. Autoclaving for a short period (5 minutes) improved the palatability of the lathyrus to a much greater degree than feed conversion efficiency (FCE) while heat treatment for longer period of time (30 minutes) resulted in the reverse pattern. A lyophilized water extract of lathyrus was growth depressing, and the nutrition quality of water extracted lathyrus was improved but was still

inferior to that of soyabean meal and wheat. Supplementation with different levels of dicarboxylic amino acids did not alleviate the growth depressing effects of lathyrus; and the lyophilized acetone extract when fed to chicks did not depress growth or induce neurotoxic symptoms. These observations implied that neurolathyrogens were not the causative agents for the poor chick performance.

In summary, it may be concluded that methionine is the first limiting amino acid in lathyrus and that lathyrus contains one or possibly two heat sensitive factors that depress appetite and nutrient utilization.

DEDICATION

This thesis is dedicated to my wife, Leenar, to my parents and sisters, and to other farmers of the world who are making a deliberate effort to produce food for the hungry world by the application of modern scientific agricultural principles.

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INTRODUCTION

A selection program has been initiated in the University of Manitoba to develop cultivars of Lathyrus sativus Linn. that are suitable for Western Canada. The agronomic potential of Lathyrus sativus Linn. is very encouraging. The crop can be grown under widely varying environmental conditions and has good yield potential. Although Lathyrus sativus Linn. has desirable agronomic characteristics, little or no information is available on its nutritional properties. It has been known for many years that Lathyrus sativus Linn. is responsible for neurolathyrism. The toxic compounds isolated from the seeds of this crop also have an effect on animals such as rat, guinea pig, cat, pigeon, chick, duck and monkey.

The objectives of this research were to assess the nutritive value of lathyrus as a feedstuff for poultry and to provide preliminary information on the nature and effect of antinutritional factors that may depress performance of lathyrus fed chicks. Also, a more up-to-date review of neurolathyrism and neurolathyrogens in Lathyrus sativus Linn. was presented to illustrate the problems one may encounter in the interpretation of experimental data.

A creamy-white seeded cultivar of lathyrus (Lathyrus sativus Linn. var. seminis albi) was used in the current feeding trials. Reports have shown that light color seeded cultivars were usually low in neurotoxins, additional beneficiary effects of this cultivar will be discussed in the literature review section.

This research should be a valuable addition to the scientific

literature regarding the evaluation of Lathyrus sativus Linn. It should also provide the basis for studies involved in the isolation and identification of the antinutritional factors in lathyrus.

LITERATURE REVIEW

A. Introduction

The genus Lathyrus consists of 130 species all with a low number of chromosomes (2n = 14), (Fouzdar and Tandon, 1975). Forty-nine species of Lathyrus have been grouped according to their common association of ninhydrin-positive compounds from chromatographic and ionophoretic analyses (Bell, 1962b). One of the species, Lathyrus sativus Linn. which causes a neurological disease in man, contains approximately 56 varieties. The different varieties of Lathyrus sativus Linn. consist of different sized seeds and colors including creamy-white, brown, grey, black and mottled (Sarma and Padmanaban, 1969; Dahiya, 1976; Roy and Rao, 1978).

Lathyrus sativus Linn., which is also referred to as "grass pea" in English, "kheshari" in Hindi and "matri" in Urdu, has other common names such as "chickling vetch" and "chick pea" (Bhagvat, 1946; Malik et al., 1967; Sarma and Padmanaban, 1969; Ghose and Haldar, 1970). In the following literature review, only the species Lathyrus sativus Linn. will be discussed. Also the term "lathyrus" will be used interchangeably with "Lathyrus sativus Linn".

Lathyrus, a member of the Leguminoceae, is cultivated in many parts of the world particularly India as a seed crop and for the purposes of fodder and green manure (Sarma and Padmanaban, 1969; Liener, 1973; Barrow et al., 1974 and Latif et al., 1975). The pulse is mostly consumed in the form of an unleavened bread (chapaties) but is sometimes eaten as paste balls or as a cooked preparation.

According to the report of Latif et al. (1975), the world production of lathyrus in 1971 was estimated by the FAO to be 6.67 million tonnes. The yields (kg/ha) of lathyrus in India (Jeswani et al., 1970) were between 420 to 700, whereas in Canada yields of lathyrus in 1978 ranged between 2700 to 3200 kg/ha (Kiehn, F., personal communication).

The agronomical characteristics of lathyrus are rather poorly documented. Lathyrus sativus Linn. is a hardy crop and survives adverse agricultural conditions. Even though lathyrus takes a longer time to reach maturity than Pisum sativus (field pea), (100-105 days to maturity for lathyrus versus 88-92 days for field pea), the rate of germination of lathyrus is faster (7 days versus 14 days). Lathyrus grows especially well under drought conditions where moisture is a limiting factor (Furgal, J., personal communication, MASCC, 1979). The average weight of 1000 grains of lathyrus is 275 grams and the average specific weight is 78 kg/100 litres. The climbing vines and leaves of Lathyrus sativus Linn. var. seminis albi plant are greyish green and the flowers are white (Furgal, J., personal communication). Various strains of Lathyrus sativus Linn are annual creeping herbs, usually with solitary, blue flowers. Average height of the plant and number of leaflets per leaf are usually different. In the species Lathyrus sativus Linn. fruits are always winged and number of seeds per fruits (pods) varies from 1 to 5 (Fouzdar and Tandon, 1975).

No insect predators or fungus disease have been observed on Lathyrus sativus Linn. var. seminis albi. Lathyrus does not have specific requirements for soil quality and climatic conditions although a cold rainy summer might be adverse to a good yield. There is no danger

of loss of grain with normal harvesting procedure (Furgal, J., personal communication). However, Roy and Bhat (1975) observed that the seeds of other varieties of lathyrus are highly susceptible to insect infestation by Callosobruchus chinensis L. (common bean weevil).

B. Gross composition of Lathyrus sativus Linn.

Analysis of the whole seeds of Lathyrus sativus Linn. have revealed the proximate composition to be: dry matter, 87 to 90%; crude protein 20 to 28%; ether extract, 0.60 to 5.20%; ash, 2.7 to 3.3%; crude fibre, 5.0 to 8.8% and carbohydrates, 46 to 58% (Rudra, 1952; Sastry et al., 1963; Malik et al., 1967; Sarma and Padmanaban, 1969; Panda et al., 1972 and Latif et al., 1975). Analysis of the total ash showed the presence of calcium (0.22 to 0.28%), phosphorus (0.26 to 0.32%), magnesium (0.11%), manganese (28 ppm), selenium (229 ppm), sodium, potassium, iron, chloride and sulphate. Sastry et al. (1963) identified β-sitosterol in lathyrus to be 1.2%. Choudhury and Rahman (1973) analyzed the ether extract (1.0%) of lathyrus and found that the ratio of unsaturated/saturated fatty acids was 2.4. Sixty-seven percent of the total fatty acids was linoleic (18:2) and 25% was palmitic (16:0). A trace amount of clupanodonic (22:5) fatty acid was also noted.

The amino acid analysis of the seeds of lathyrus after acid hydrolysis were performed by Sarma and Padmanaban (1969) and Latif et al. (1975). The results (Table 1) showed that the seed is rich in lysine and contains relatively adequate concentrations of the other essential amino acids except cystine and methionine. Tryptophan contents in different varieties of Lathyrus sativus Linn. ranged from 0.09 and 0.23% on a dry

Table 1. The amino acid composition of *Lathyrus sativus* L.[a]

Amino acid	Reference	
	Sarma and Padmanaban (1969)[b]	Latif et al. (1975)[c]
Lysine	1.85	1.12
Histidine	1.10	0.77
Arginine	1.41	1.68
Aspartic acid	1.80	2.34
Threonine	0.85	0.71
Serine	1.20	-
Glutamic acid	2.25	3.67
Proline	1.46	0.84
Glycine	0.74	0.94
Alanine	0.80	0.88
Cystine	Trace	0.75
Valine	0.81	1.07
Methionine	0.35	0.07
Isoleucine	1.01	0.94
Leucine	1.45	1.63
Tryrosine	0.62	0.66
Phenylalanine	1.03	0.89
Asparagine	-	1.57
Total	18.73	20.53

[a]All values are expressed as % of sample.

[b]The crude protein content was estimated to be 24.5% (Nx6.25).

[c]The values are converted from g/kg. The crude protein content was estimated to be 27.38% (Nx6.25).

weight basis (Chekalin and Krasnaya, 1972). These differences in tryptophan content may be attributed to cultivar and/or environmental variations.

The gross caloric value of lathyrus was reported by Panda et al. (1972) to be 16.39 MJ/kg. Latif et al. (1975) calculated the metabolizable energy of lathyrus to be 11.29 MJ/kg.

C. Lathyrus sativus Linn. as an animal feed ingredient

Very little research has been reported on the feeding value of lathyrus for animals. Most previous studies were designed to assess the neuropathological effects of lathyrus rather than its nutritional properties.

Malik et al. (1967) reported an increase in chick growth depression which was almost proportional to the levels of lathyrus supplemented in the ration. There was a significant growth depression and pancreatic hypertrophy (expressed as per unit body weight) in chicks fed diets containing 25% lathyrus when compared to chicks fed maize-sesame cake control and 5, 10, 15 and 20% lathyrus diets. Feed consumption seemed to increase with increased levels of lathyrus except in chicks fed the 25% lathyrus supplemented diet but these differences were not statistically significant ($P<0.05$). Moreover, Malik et al. (1967) found the dressed body weight of the chicks fed 25% lathyrus to be significantly ($P<0.05$) less as compared to the control and other diets containing lesser amounts of lathyrus during the eight-week experimental period. In contrast, Latif et al. (1975) showed that chicks fed on lathyrus diets for a two-week period grew more slowly than chicks on the the maize-soya diet but higher percentages of lathyrus (22 and 37%) did not depress growth rate any

more than the 10% level. There were also significant ($P<0.05$) decreases in feed consumption with increased amount of lathyrus in the diet, but FCE was better in the case of the chicks receiving 37% lathyrus in their diets than those fed on 10 and 22%. Contradictory results were obtained by Malik et al. (1967) who reported a significant ($P<0.05$) decrease in FCE with chicks fed 25% lathyrus in their diets compared to those receiving 0, 5, 10, 15 and 20%.

Methionine supplementation of a diet containing 25% lathyrus improved FCE of chicks to that of the control group (Malik et al., 1967), whereas when the diet contained only 10% lathyrus no improvement was observed (Latif et al., 1975). Latif et al. (1975) also reported that there was a positive response to L-tryptophan supplementation, but growth and feed intake were still inferior to chicks fed the maize-soya control diet.

Malik et al. (1967) showed that neither FCE nor hypertrophy of the pancreas of chicks fed autoclaved or water-treated lathyrus was significantly ($P<0.05$) different to those fed a diet containing the same concentration (25%) of raw lathyrus. There nevertheless was a significant ($P<0.05$) improvement in dressed weight of birds fed a water-treated, autoclaved or methionine suppplemented lathyrus diet as compared to those fed the raw lathyrus. In contrast, Latif et al. (1975) reported that both autoclaved and micronised lathyrus (37%) fed birds showed significant ($P<0.05$) improvements in feed consumption and body weight gain as compared to those fed on raw lathyrus. These improvements resulted in growth rates similar to that obtained with the maize-soya control group. Moreover, feed conversion efficiency in groups fed

autoclaved or micronised lathyrus diets were significantly ($P<0.05$) better than the maize-soya control group which was the same as those fed on raw lathyrus.

The findings of Malik et al. (1967) suggested that high level (25%) of lathyrus in broiler chick diet caused a significant ($P<0.05$) decrease in body weight gain and FCE. These antinutritional effects could be alleviated by DL-methionine supplementation but neither water-treated nor autoclaved lathyrus improved chick performance as compared to those of the maize-sesame cake control group. These observations might suggest that the deleterious effects of lathyrus were mainly created by amino acids imbalance, whereas in the report of Latif et al. (1975) feed conversion efficiency did not seem to be reduced in any of the lathyrus fed birds. Lathyrus when fed to chicks depressed feed consumption and subsequently weight gain. It may therefore by hypothesized from these latter results that there is an appetite depressing factor in lathyrus which can be reduced by either autoclaving of micronising (Latif et al., 1975). Also it appears that lathyrus is deficient in either methionine and/or tryptophan. Moreover, the apparent differences found between the reports of Malik et al. (1967) and Latif et al. (1975) might be explained by the possibility of the use of different varieties of Lathyrus sativus Linn. Subsequent analysis demonstrated that the content of the lathyrus neurotoxin varied from 0.142 to 0.680% and variations in trypsin inhibitory activities were also observed in different cultivars (Roy and Bhat, 1975). On the other hand, the difference might be caused by the use of different basal diets (maize-soya versus maize-sesame cake) and the length of the experimental period (2 weeks versus 8 weeks).

With the use of white leghorn chicks, Panda et al. (1972) demonstrated retarded growth followed by 100% mortality when the diet contained 50% lathyrus. Guinea pigs fed a mixed diet consisting of 30% lathyrus and 50% wheat did not develop alopecia, dermatitis and deep trophic ulcers in the hind legs whereas these symptoms developed in groups fed diets containing 50, 75 and 80% of lathyrus. Also the same deleterious effects occurred in guinea pigs when they were fed either whole or dehulled lathyrus (Bhagvat, 1946). In the case of feeding 50 to 100% powdered lathyrus to rats, neither external toxic symptoms nor any pathological abnormalities in the internal organs were observed (Sastry et al., 1963). Also there was an increase in feed consumption but a decrease in body weight gain in groups fed a diet containing whole lathyrus powder (95%) and 5% ground nut oil. Moreover, digestibility of crude protein of rats fed the whole lathyrus diet was the highest with respect to the maize-wheat control diet and diet containing 50% powdered lathyrus (Sastry et al., 1963). The percentages of the nitrogen digested for the 0, 50 and 100% lathyrus diets were 58, 61 and 75%, respectively. The rats in all groups maintained a positive balance of nitrogen, calcium and phosphorus during the metabolism trial conducted over a three-day period. The above findings fall in line with the literature of Stockman (1929) cited in Barrow et al. (1974), which reported that there were species differences in their susceptibility to lathyrus toxicity. However, the use of different varieties of lathyrus might also create different effects. Further evaluations are needed to establish the feasibility of using lathyrus as an animal feed ingredient.

D. Neurolathyrism and neurolathyrogens

a) Neurolathyrism

The association between the consumption of large amounts of Lathyrus sativus Linn. and neurolathyrism in man has been known from the time of Hippocrates. Neurolathyrism, the term which was coined by Selye (1957), refers to neurological disorders in man that occur following the prolonged consumption of lathyrus. Symptoms of the onset of the disease are spastic paraplegia and degenerative pathological changes in the spinal cord.

Reviews of certain aspects of neurolathyrism are available (Stockman, 1929; Selye, 1957 and Barrow et al., 1974). A detailed history and clinical manifestations of neurolathyrism in man were described in Barrow et al. (1974). Major outbreaks of neurolathyrism in India occurred during periods of famine either caused by severe hailstorms and rains, blight or drought. As mentioned previously, lathyrus is a hardy crop which thrives with great luxuriance during periods of drought. It therefore becomes a dietary staple during periods of famine. The use of lathyrus at a level from one-third to one-half of the diet for two or three months has resulted in the onset of the neurotoxic disease. Although not all persons consuming the pulse are affected, adult males are said to be more frequently affected than young males and females (Barrow et al., 1974).

According to a epidemiological study of neurolathyrism in parts of India, the prevalence of lathyrism is associated with social status since the number of lathyrism cases rose as individual income decreased

(Dwivedi and Prasad, 1964). Analysis of 250 lathyrism cases showed that 56% were in the "latent" form and 44% were in the fully developed or "established" form of the disease. The earliest symptoms are myospasm in calf muscles, which appear 10 to 15 days prior to the onset of the disease, and are observed in both forms of the disorders (Dwivedi and Prasad, 1964).

b) Isolations, chemical syntheses and biosynthesis of neurotoxins responsible for neurolathyrism

Several toxic substances have been isolated from the seeds of Lathyrus sativus Linn. (Rao et al., 1964; Murti et al., 1964; Bell and O'Donovan, 1966; Rukmini, 1968 and 1969; Harrison et al., 1977). Methods of chemical synthesis and biosynthesis of some of these compounds have also been carried out by several groups of workers (Rao et al., 1964; Malathi et al., 1967 and 1970; Mehta et al., 1972; Rao, 1975; Haskell and Bowlus, 1976; Wu et al., 1976 and Harrison et al., 1977). Each of the known compounds will be discussed in the following sections.

1) β-N-oxalyl-L-α, β-diaminopropionic acid (ODAP) and β-N-oxalyl-D-α, β-diaminopropionic acid (OD'AP)

ODAP is one of the compounds that can be extracted with different concentrations of alcohol:water from the seeds of lathyrus. Its structural formula has been calculated to be $C_5H_8O_5N_2$. The compound is highly acidic in character and forms oxalic acid and L-α, β-diaminopropionic acid (DAPA) on acid hydrolysis (Rao et al., 1964; Murti et al., 1964; Nagarajan et al., 1965 and Mehta et al., 1972). The yields of ODAP as a percent of the whole seed, after recrystalization from hot distilled water, vary from 0.5 to 1.0%. The melting point reported by

Murti et al. (1964) was 174-175°C (with gas evolution) whereas the value reported by Rao et al. (1964) and Mehta et al. (1972) was 206°C. ODAP also has a specific rotation of -36.9° (C, 0.66, 4NHCl) and apparent pK values of 1.95, 2.95 and 9.25 corresponding to the two carboxyl and one amino functions respectively. A specific rotation of -28.1° (C, 1.99; 5NHCl) and -19.5° (C, 2.72; 4NHCl) was obtained by Murti et al. (1964) and Haskell and Bowlus (1976) respectively.

ODAP was first synthesized by Rao and coworkers (1964) using an aqueous methanolic solution of a copper complex of DAPA prepared at pH 4.5-5.0 with dimethyl oxalate under controlled pH conditions, and the final compound was isolated by ion exchange chromatography (Dowex 50-H^+ column) after precipitating the copper. Later on, Rao (1975) proposed another method in which L-aspartic acid, the starting material was reacted with sodium azide in 30% fuming sulfuric acid. L-α, β-diaminopropionic acid hydrochloride, which was isolated in yields greater than 75%, was then reacted with potassium methyl oxalate to form ODAP. Moreover, OD'AP was synthesized when D-aspartic acid was the starting material. The specific rotation of this compound is +28° (C, 2.0; 0.5NHCl). In the preliminary experiments, Harrison et al. (1977) found that the extent of oxalylation by a number of reagentswas very low with Cu^{++} present at pH 5 (as suggested in Rao et al. (1964)), whereas in the absence of Cu^{++}, diethyl oxalate gave a quantitative conversion of DAPA to a mixture of oxalyl derivatives. The extent of the reaction was markedly pH sensitive, being greater at pH 10 than at any higher or lower pH value. Harrison et al. (1977) suggested that the formation of an uncharged amino group at about pH 10 encouraged migration of the

oxalyl group, since the isomerization of the N-oxalyl isomers was found to be maximal at pH 10.

The chemical procedures for the synthesis of ODAP, using copper chelate of DAPA and various oxalate esters, was said to be economically prohibitive on any reasonably large scale owing to poor yields and the high costs of the starting materials. The new method proposed by Haskell and Bowlus (1976) starts with L-asparagine which is converted to L-β-amino-α-(p-toluene sulfonyl) amino-propionic acid via the Hoffmann degradation reaction of p-toluenesulfonyl-L-asparagine. A moderate yield between 45-50% of ODAP was obtained by this method.

The pathway for the biosynthesis of ODAP has also been established. Malathi et al. (1967) initially demonstrated that the synthesis of ODAP can proceed by condensation of oxalyl-CoA with the β-amino group of DAPA, when lathyrus seeds were germinated in the presence of labelled oxalic acid and DAPA. Malathi et al. (1970) subsequently reported that the biosynthetic pathway involves two reactions. The first reaction is catalysed by oxalyl-CoA synthetase which has properties similar to that of the enzyme in Pisum sativus (field pea). The second reaction is catalysed by another enzyme, oxalyl-CoA-α, β-diaminopropionic acid oxalyl transferase (ODAP synthetase), which is specific to Lathyrus sativus Linn. ODAP synthetase has been purified and partial resolution of the enzymatic activities of the two enzymes has been achieved using CM-sephadex columns (Malathi et al., 1970).

ODAP is suggested to be the principle causative agent in neurolathyrism, but the mechanism of action is still unclear and it is uncertain whether neurolathyrism is caused by the compound, ODAP, alone.

2) α-N-oxalyl-L-α, β-diaminopropionic acid (L-ODAP)

Other than ODAP and OD'AP, the α-isomer of ODAP, namely α-N-oxalyl-L-α, β-diaminopropionic acid (L-ODAP) has also been isolated from Lathyrus sativus Linn. (Bell and O'Donovan, 1966; Harrison et al., 1977). L-ODAP generally constitutes between 4-10% of the total toxin fraction during the isolation of ODAP (Rao, 1975; Wu et al., 1976).

Chemical synthesis of L-ODAP has also been performed by Wu et al. (1976) and Harrison et al. (1977). The method described by Wu et al. (1976) involved the condensation of L-β-benzyloxycarbonyl-amino-α-aminopropionic acid and oxalyl chloride followed by the hydrogenolysis of the β-amino protecting group. The crude product, containing predominantly L-ODAP and small amount of ODAP, was purified by repeated washings with dimethyl oxalate and water to obtain the pure form of L-ODAP

The procedure used by Harrison et al. (1977) was different from that of Wu et al. (1976). L-α, β-diaminopropionic acid monohydrochloride was first reacted with diethyl oxalate and the mixture was maintained at pH 10 using saturated lithium hydroxide. After a period of about 2 hr at 30°C, the mixture was evaporated to dryness at 40° in vacuo. The dry material was resuspended in water, adjusted to pH 10 using the lithium hydroxide solution and heated at 80°C for 17 hr. Thereafter, the solution was desalted, after cooling, by passing through a column of Zeokarb 225 (8% cross linked, pyridinium form). The elutant, which contained both L-ODAP and ODAP, was further separated, freeze-dried and purified. The yield of L-ODAP and ODAP from 1 gm of DAPA was 0.54 g and 0.31 g, respectively.

3) N-β-D-glucopyranosyl-N-α-L-arabinosyl-α, β-diaminopropionitrile (DAPN)

A water soluble aliphatic amino acid glycoside with a nitrile group has also been isolated from the aqueous extracts of Lathyrus sativus Linn. (Rukmini, 1968 and 1969). The chemical structure of the compound was assigned on the basis of chemical and physical evidences, as N-β-D-glucopyranosyl-N-α-L-arabinosyl-α, β-diaminopropionitrile (DAPN). Its molecular composition is $C_{14}H_{25}O_9N_3 \cdot H_2O$. Acid hydrolysis of the compound with 6N HCl for 8-9 hr at 100°C yields arabinose and glucose, and alkaline hydrolysis of the glycoside with 4N NaOH for 4 hr at 80°C gives DAPA.

c. Biological effects of natural and chemically synthesized neurotoxins

When ODAP was injected into day-old chicks at 500-600 mg/kg body weight, it caused stiffening of the neck, head retraction, convulsions and extensor paralysis of the hind limbs (Adiga et al., 1963; Rao et al., 1964). At 350 mg/kg body weight, ODAP induced similar symptoms with a lesser degree of severity (Lakshmanan et al., 1971). Whereas at higher doses, such as 750-800 mg/kg body weight, chicks developed complete paralysis at 4 hr and recovered from the treatment after a period of 12 to 24 hr (Jacob et al., 1967). Unlike ODAP, the naturally occurring neurotoxin, OD'AP (the D-isomer) was not neurotoxic when administered intraperitoneally even at a high dose of 11 g/kg body weight (Rao, 1975). With the L-isomer of ODAP, no neurotoxic symptom was observed when 300 mg and 600 mg/kg body weight were injected into day-old chicks (Wu et al., 1976). Harrison and coworkers (1977) also reported that high doses of 660 to 880 mg/kg body weight, when injected

intraperitoneally into day-old chicks, did not induce any convulsant activity. On the other hand, some birds which received 900-1000 mg/kg body weight developed convulsions within 3 hr but subsequently recovered (Harrison et al., 1977). They suggested that the toxicity of L-ODAP at high doses was probably due to its structural relationship to oxamic acid. Alternately, isomerization of high doses of α-isomer to β-isomer might occur in vivo, producing sufficient ODAP to induce convulsions. Harrison et al. (1977). Rukmini (1968 and 1969) have isolated another neurotoxin from lathyrus, DAPN, which induced paralysis of the limbs in day-old chicks. The symptom appeared within 5 to 10 minutes of intraperitoneal injection of 500 mg DAPN/kg body weight.

ODAP has also been shown to cause chronic ammonia toxicity in young and acidotic adult rats when administered intraperitoneally at 180 to 330 mg/kg body weight (Cheema, Padmanaban and Sarma, 1969, 1970, 1971a and 1971b). The neuroexcitatory properties of ODAP have been reported to be more potent than DL-homocysteate and only slightly less than N-methyl-D-aspartate (Johnston, 1973). ODAP also induced lesions in the retina of infant rats, which was very similar, if not identical, to the lesion seen after treatment with glutamic acid (Olney et al., 1976). When 500 mg/kg body weight of ODAP was injected into 3-day-old mice, animals assumed a characteristic hunched posture, suffered severe muscle spasms, gasped for breath and died within 30 minutes. An equivalent dose of L-ODAP produced no symptom and was not fatal (Wu et al., 1976). Hypoglycemia, hypercholesteremia and hyperproteinemia were obtained in rats given small doses of toxic extract of lathyrus for 5 weeks, but no death was observed (Bhadra et al., 1973). Vadlamudi and coworkers (1973)

found that both the seeds and pulses (dehulled seeds) of lathyrus contained the neurotoxic active principle. The toxic principle from both fractions produced acute poisoning and were highly toxic to rats causing death by respiratory failure.

From in vitro studies with mitochondria isolated from bovine brain, Duque-Magalhaes and Packer (1972) showed that ODAP affects the metabolism of isolated brain mitochondria through: a) an apparent inhibition of respiration with glutamate, glutamine and α-ketoglutarate; b) inhibition of glutamate transport across the inner mitochondrial membrane through competitive binding; and by c) apparent activation of mitochondrial glutaminase. ODAP also depressed growth of wild type or of amino acid requiring strain of Saccharomyces cerevisiae (common baking yeast). But the growth depression was alleviated by either L-glutamate or L-aspartate supplementation in the growing media (Mehta et al., 1972).

ODAP was also found to produce neurological symptoms in ducklings and baby pigeons (Nagarajan et al., 1965). The dosage employed to produce the symptoms were 60 to 70 mg for ducklings weighing 45 to 50 g, and 150 to 200 mg for baby pigeons weighing 100 to 120 g. Parenteral administration of ODAP to young rats, mice, guinea pigs and monkeys at dosages from 200 to 1000 mg/kg body weight produced no visible signs of neurotoxicity (Nagarajan et al., 1965). On the other hand, adult monkeys developed spastic paraplegia after 20 mg of ODAP intrathecal injections. Destruction of the nerve cells of the grey matter of the spinal cord accompanied by proliferation of microglial cells were observed (Rao et al., 1967; Mani et al., 1971). Neurotoxic symptoms such as ataxic gait,

sudden jerking movements, dragging of the legs or a hopping movement and rigidity of the neck were also induced by intraperitoneal injections of ODAP into a 12-day-old dog (dose 182 mg/kg body weight) and into an 8-day-old guinea pig (dose 357 mg/kg body weight) (Rao and Sarma, 1967).

Although intraperitoneal administration of large doses of ODAP (0.5 to 1.0 g) to monkeys weighing 1.5 to 2.0 kg produced no ill effect, a single intraperitoneal injection of 10 ml of crude alcohol extract of lathyrus (equivalent to 5 or 6 g of seed per ml) to these animals caused drowsiness and sometimes, stupor within 30 minutes after injection. These animals recovered slowly. Higher doses of the extract (15 to 20 ml) resulted in coma and death (Nagarajan et al., 1965). Moreover, daily intraperitoneal injections of concentrates of the alcoholic extracts into chicks, at 2 ml/kg body weight for 4 weeks, resulted in skeletal deformities, which particularly affected the sternum (Nagarajan et al., 1965).

Other than the effects of the lathyrogens in animals, Siddiq et al. (1975) showed the acute (single) treatment of the plant system either with ODAP or crude extracts of lathyrus did not produce a marked effect on the mitotic behavior, however, physiological damage, as reflected by suboptimal germination and growth rates, was observed.

d. Mode of action of the neurotoxins

Although the Lathyrus sativus Linn. neurotoxin, ODAP, is believed to cause neuropathological disorders in humans, there is practically no evidence regarding its mode of action in humans. From the findings of different groups of scientists using various experimental animals, the mode of action of the neurotoxin could be categorized into "neuroexcitatory"

and "neurotoxic" effects.

1) Neuroexcitatory effects of neurolathyrogens

ODAP resembles glutamic acid in molecular structure and is a neuroexcitant and convulsant similar but more potent than glutamate (Duque-Magalhaes and Packer, 1972; Mehta et al., 1972 and Olney et al., 1976). Glutamate is a precursor of the inhibitory transmitter candidate γ-aminobutyric acid (GABA), and high levels of glutamate in the cerebral cortex of the mammalian system has been shown to be related to the functional integrity of the cortex (Johnson, 1972). ODAP has been reported to cause lesions in the retina and brain of infant rats (Olney et al., 1976). These lesions consisted of acute swelling of dendrites and cell bodies of neurons, with degenerative changes occurring rapidly in both intracytoplasmic organelle systems and neuronal nuclei. Moreover, lesions induced by ODAP were typically different than the patterns of damage induced by glutamate. Olney et al. (1976) suggested that the difference in effects might be due to dissimilar processing of the two compounds by blood brain barrier (BBB) or differential binding at receptors within affected brain regions.

Rao and Sarma (1967) showed that ODAP did not induce any neurotoxic symptoms in normal adult birds and rats at doses that caused neurological disorders in day-old chicks and young rats (10-12 days old). They proposed that the innocuous nature of ODAP to adult animals could be due to an effective BBB system. Treatment of adult animals with acid-forming salts like calcium chloride or ammonia chloride, or drugs like Diamox (acetazoleamide), sulphanilamide or salicylic acid, all of which

are known to cause an "acidotic" state, was found to make adult animals susceptible to small doses of ODAP upon intraperitoneal administration (Rao and Sarma, 1967). They suggested that the increase in susceptibility in these animals might not be due to the increased permeability under "acidotic" conditions, but rather due to the changes in various enzyme activities resulting in alterations of levels of certain metabolites. These metabolites probably in turn alter the permeability of the toxins, and thus lower the threshold of susceptibility.

Several groups of workers were able to recover unchanged labelled ODAP from brain tissues or urine of intoxicated animals with mature BBB (Mehta et al., 1976; Rao, 1978a and Parker et al., 1979). On the other hand, Cheema et al. (1971a) reported the recovery of the keto acid product of the transamination reaction between ODAP and α-ketoglutarate (α-kg) in the total keto acid fractions of liver and kidney of rats. The keto acid product of this transamination reaction has been identified as β-N-oxalyl-α-keto-β-amino-pyruvic acid. Studies of the properties of the ODAP transamination reaction revealed that aspartate-α-kg transaminase and the enzyme catalysing transamination between ODAP and α-kg had similar properties (Cheema et al., 1971a). The enzyme requires pyridoxal phosphate and functional sulphydryl groups for its activity, and has a pH optimum in the range of 7.0-7.8. A temperature optimum of around 50°C was also obtained and metal ions are not required for the enzyme activity (Cheema et al., 1971a).

The neurotoxin was detected in the Central Nervous System (CNS) of the acidotic monkey and also in the normal monkey in nearly the same

quantity. The neurotoxin was largely localized in the lumbosacral region of the spinal cord (Rao, 1978a). Drowsiness, vomiting, muscle tremors, twitching, convulsions and death occurred in young male squirrel monkeys following injection of 750 and 2000 mg ODAP/kg body weight. Electroencephalographic changes characteristic of each stage of intoxication were observed (Parker et al., 1979). The concentrations of the neurotoxin 90 minutes after injection in the CNS of the adult rat and the day-old chick were almost the same and increased 2 fold by 24 hr (Rao 1978a), whereas Mehta et al. (1980) investigated the rate of loss of labelled ODAP from animal tissue and did not observe increase in total concentrations of ODAP in CNS after 24 hr. However, the relative concentration of radioactivity (dpm/g) in cerebellum was shown to increase with time relative to the brain as a whole (Mehta et al., 1980). Moreover, the half-life for disappearance of radioactivity from cerebellum (32.7 hr) matched that for bone and was the longest observed in the experiment. ODAP was also detected in significant concentrations (Lakshmanan and Padmanaban, 1977) in synaptosomal fractions isolated from the brain of young rats and the spinal cord of adult monkeys in animals that developed neurotoxic disorders after ODAP administration. However, isolated synaptosomes fail to exhibit a transport system for ODAP uptake. ODAP administered in vivo appears to be localized in a population of synaptosomes which has a high capacity for the uptake of glutamate (Lakshanan and Padmanaban, 1977). The amount of labelled-ODAP in the CNS following its intraperitoneal injection was twice that seen following its intravenous injection, and species (and age) differences in susceptibility of the Lathyrus sativus neurotoxin was

independent of its entry into the CNS (Rao, 1978a).

In vitro studies showed that ODAP inhibited glutamate uptake (15-20%) by synaptosomes isolated from both young rat brain and monkey spinal cord (Lakshmanan and Padmanaban, 1974). With the high affinity uptake system of synaptosomes, inhibition of glutamate uptake even at 15 to 20% could be significant because of the extremely low concentration of glutamate required to cause excitation. Lakshmanan and Padmanaban (1974) also mentioned that ODAP inhibits glutamate uptake but not glycine uptake in monkey spinal cord synaptosomes. Since glycine is an inhibitory transmitter at the spinal cord level, the above observation could mean that the effects of ODAP in causing convulsions in young rats and hind leg paralysis in monkeys are primarily neuroexcitatory, and are possibly mediated by glutamate (Lakshmanan and Padmanaban, 1974). Similarly, inhibition of glutamate transport across the inner mitochondrial membrane of bovine brain in vitro was reported by Duque-Magalhaes and Packer (1972). They showed that ODAP exerts its effect through fully competitive binding - presumably to the glutamate carrier present in the mitochondrial membrane. ODAP depressed growth of wild type of Saccharomyces cereviscae has also been reported (Mehta et al., 1972). The toxin behaved as a competitive inhibitor of the transport of L-glutamate and L-asparatate into the resting yeast cells, but it did not inhibit the incorporation of these amino acids into aminoacyl-t RNA or into protein. Growth depression was alleviated by supplementing the medium with L-glutamate or less effectively with L-aspartate. Sensitivity of various yeast strains to the toxin is

inversely related to the size of the amino acid pool, of which glutamic acid is a major component (Mehta et al., 1972).

Goswami (1973a) observed that ATPase activity is comparatively high in animals treated with ODAP. This increased ATPase activity in the toxin treated animals may be due to direct activation of the ATPase system or at least in part it may be due to interference of ion transport as ATPase activity is connected with the mechanisms responsible for ion transport. Goswami (1973a) suggested that the sodium and potassium ions that activate the ATPase are also the ions that are pumped across the membrane, and they possibly can act in a better way through the active site for the enzyme due to the presence of ODAP and DAPN, or it may be that the toxins are linked up with the enzyme through some other sites and thereby activate the active sites of the enzyme for sodium and potassium ions. The initial increase of ATPase activity may also be a temporary compensatory mechanism of the degenerating tissue (Goswami, 1973a). Subsequently, Goswami (1973b) was able to show marked increases of potassium, sodium and water in the spinal fluid and cerebral cortex tissues of cats receiving prolonged administration of Lathyrus sativus toxins in comparison to the tissues from untreated groups. He proposed that the retention of electrolytes (Na^+ and K^+) and water in the brain might be responsible for hydration of the nerve cells and myelin sheaths resulting in demyelination which might explain the spastic paraplegia in neurolathyrism. It has also been reported that ODAP is a powerful excitant of spinal interneurones and betz cells of the cat spinal cord (Watkins et al., 1966). Cheema et al. (1970) reported that brain homogenates prepared from ODAP injected animals

showed a higher rate of respiration. There was a decrease in the brain glucose, glycogen, ATP, phosphocreatine and acetylcholine levels of the convulsing animals. Also, the inorganic phosphate, lactic acid and acetylcholinesterase levels increased. The above findings established that ODAP is a typical convulsant. Johnston (1973) found that ODAP, as excitants of feline central neurones, was more potent than DL-homocysteate and only slightly less active than N-methyl-D-aspartate, whereas L-aspartate, D- and L-glutamate are weaker excitants. These results suggest that the excitant amino acids directly depolarize the central neurones and that this effect can be counteracted by injections of amino acids (GABA and glycine) which have a direct hyperpolarizing action on neurones.

The period of complete paralysis of chicks after intraperitoneal injection of ODAP has also been associated with a fall in the activity of the glutamate dehydrogenase. This decrease was followed by a restoration to normal level during clinical recovery from paralysis (Jacob et al., 1967). In contrast, in vitro studies showed no change in glutamate dehydrogenase but a significant decrease was found in L-glutamate-1-carboxylase (GAD). Jacob et al. (1967) proposed that the difference might be caused by differential susceptibility of the subcellular fractions of the brain to the toxin administered in vivo and in vitro. It is possible that the paralysis observed in chicks might be due to acute cerebral seizures provoked by dearrangement of the natural balance between glutamic acid and GABA. The reasoning is that GAD is the enzyme responsible for the decarboxylation of glutamic acid into GABA. Decreased GAD activity (as mentioned in Jacob et al., 1967)

resulted in a decrease of GABA, a substance which is important for the action of inhibitory neurones. A failure of regulation of the excitatory neurones possibly results in an uncontrolled spreading of electrical impulses and in seizures (Wiechert and Herbst, 1966). Since acid hydrolysis of ODAP in vitro produced oxalic acid and DAPA, treatment of animals with acid-forming salts could possibly induce ODAP hydrolysis in vivo. However, neither oxalic acid nor DAPA, at comparable levels to ODAP, produced any visible symptoms (Rao et al., 1964). Rao (1978b) has developed a sensitive and specific colorimetric method for the determination of DAPA and ODAP, and indicated the presence of an alkali labile metabolite of DAPA in the liver and kidney of the ODAP treated animals. Further research is needed to substantiate the mode of action of these metabolites.

2) Neurotoxic effects of neurotoxins

Other than being an excitant amino acid, ODAP might also influence other reactions in glutamate metabolism. Gradual decreases of the activities of serum glutamic oxalylacetic and glutamic pyruvic transaminases with time was observed in albino rats treated with ODAP. The decrease of these enzymes correlated with decreased liver protein levels and inhibition of trypsin (Goswami and Dastidar, 1972). Brain glutamine concentrations in treated animals showed striking increases with time compared to the steady but small increase in blood glutamine concentration (Cheema et al., 1969). Since glutamine formation is a major mechanism for the detoxification of ammonia in the brain, ODAP must have caused a significant increase in ammonia concentration in the brain of the experimental animals. The increased levels of ammonia in ODAP

treated animals was proposed by Cheema and his coworkers (1969) to be due to an interference with ammonia-generating or ammonia-fixing mechanism in the brain rather than the catabolism of ODAP. This was supported by observations of Cheema et al. (1971b) who found markedly increased levels of acid protease and transglutaminase in the brain of ODAP-treated animals, whereas the activity of glutamate dehydrogenase was slightly reduced. The mechanism of the sudden increase in the degradative enzyme activity soon after ODAP administration is not clear. It is unlikely that de novo synthesis of the enzymes have taken place in such a short time (Cheema et al., 1971b). It is a possibility that the neurotoxin, ODAP, might have damaged the lysosomal membrane leading to the release of the degradative enzymes which can account for generalized increase in the catabolism of macro-molecules (Cheema et al., 1971b, Lakshmanan et al., 1971).

In conclusion, the mechanism of action of neurolathyrogen in humans could be a combination of both neuroexcitatory and neurotoxic behaviour. Other growth inhibitors, found in Lathyrus sativus, might amplify the severity of the symptoms leading to irreversible paralysis in the case of neurolathyrism in man. Parenteral administration of Lathyrus sativus toxins in albino rats was found to cause overall stimulation of the hemopoietic system which might be an adaptive mechanism (Adhya et al., 1975). This might possibly explain the reversibility of experimental lathyrism in rats and chicks.

E. Other growth inhibitors found in Lathyrus sativus Linn. and their effects on animals

Selenium was suggested to be the toxic substance of Lathyrus sativus

Linn. responsible for lathyrism (Rudra, 1952). Lathyrus sativus was found to contain 229 ppm of selenium whereas 12 ppm was obtained in Pisum sativus. The mode of action of selenium was reported to be its interference of methionine metabolism. Motor neurone lesions in lathyrism patients were healed when methionine was given with vitamin B_{12}, while methionine given alone has no effect (Rudra, 1952).

L-homoarginine has been isolated and characterized from the seeds of Lathyrus sativus (Rao et al., 1963). Successive intrathecal administration of L-homoarginine resulted in large central cavitations of lumbosacral cord with myelomalacia, vascular proliferation and reactive polymorphs, gitter cells and astrocytes. The rest of the CNS was normal (Mani et al., 1971). L-homoarginine might be a natural precursor to lathyrine, which is formed by cyclization and dehydration. The difference between species of lathyrus containing lathyrine and those containing only homoarginine is the presence or absence of an enzyme system capable of bringing about this transformation have also been proposed (Bell, 1962a and Rao et al., 1963).

The presence of antitryptic activity in water extracts and extracts of phosphate buffer of Lathyrus sativus was first demonstrated by Roy and Rao (1971). The partially purified trypsin inhibitor induced growth depressions and increased percentage of pancreatic weights to body weights in rats (Roy, 1972a). Further purification, fractionation, and characterization of properties of trypsin inhibitor isolated from Lathyrus sativus was also performed (Roy, 1972b). Even though trypsin inhibitor induced growth depression, administration of ODAP with or without trypsin inhibitor isolated from Lathyrus sativus seeds did not influence the

neurological manifestations due to the neurotoxin (Roy, 1973).

Phytohemagglutinins (PHA) or lectins, which have the ability to agglutinate red blood cells, was determined in lathyrus and compared with values for other legumes (Latif et al., 1975). Lathyrus sativus Linn., has PHA titre between 1:100 and 1:1000 and is more toxic than soyabeans with a titre of about 1:10. Raw jackbeans with a PHA titre of about 1:20,000 are far more toxic than navy beans (PHA titre of about 1:10,000) and lathyrus. Latif et al. (1975) showed that heating raw lathyrus to 160°C for only 30 seconds or to 121°C for 5 minutes could eliminate its growth depression properties. It may be assumed that most proteins including PHA and trypsin inhibitor were denatured under these conditions.

Other compounds like cyanin (cyanidin, 3:5 diglucoside) and pelargonin (pelargonidin, 3:5 diglucoside) have also been identified, on the basis of Rf values, from the methanol:hydrochloric acid extracts of lathyrus (Sarkar and Banerjee, 1977). These compounds may also be growth inhibitors.

F. Screening for low toxicity lines

ODAP has been reported as the most probable principle responsible for neurolathyrism in man. The approach for the eradication of lathyrism will be to develop, through plant breeding, varieties of Lathyrus sativus which are inherently low or completely free of their ODAP content (Jeswani et al., 1970). Sarma and Padmanaban (1969) have reported that Lathyrus sativus consists of about 56 varieties, among which the levels of ODAP in ten varieties examined by Roy and Bhat (1975) was found to be between 0.142 to 0.680%. Neurotoxin content as high as 1.85% in

commercially grown strain (Rewa-2 (M.P.)) has also been reported by Jeswani et al. (1970).

Over 1500 samples of Lathyrus sativus were screened for their ODAP content by paper chromatography. A few varieties having a low neurotoxin content (0.15 to 0.30% ODAP) were isolated (Jeswani et al., 1970). From these low neurotoxin lines further single selections were followed in the succeeding generation to find out the range of variability of the ODAP content. The results obtained by Jeswani et al. (1970) showed that the ODAP content of the selections is much lower than some of the commercially grown strains, and the yield potential of some of these new lines is quite comparable to and, in some cases, even greater than the commercial types.

The chemical estimation of ODAP is quite laborious and expensive which puts a limit on screening and further identification of low neurotoxin lines. Morphological characters of plant or seed which serve as markers for quality traits are of particular importance to plant breeders and have always been preferred to chemical methods for preliminary evaluation of plant material have been proposed.

Dahiya (1976) found the cultivars with early maturity, smaller seeds of light cream colour contain low levels of ODAP, whereas Roy and Rao (1978) showed that biochemical parameters, including levels of ODAP, trypsin inhibitory activity, protein, moisture and ether extractable fraction, did not have any significant correlation of considerable magnitude with either color and size of 29 varieties of Lathyrus sativus. The differences between these authors are not clear. Roy and Rao (1978) suggested that they might be due to differences in the sample size,

maturity of seeds, soil condition and varieties of seeds investigated by size and color. A further study with more varieties may be of interest to supplement the findings on chemical characteristics.

Studies on susceptibility to insect attack in varieties of Lathyrus sativus seeds have been performed by Roy and Bhat (1975). When the seeds were exposed to insects all ten varieties were affected, but the varieties with low trypsin inhibitor content showed some protection against infestation. One variety (P-24) with low ODAP and trypsin inhibitor content was found to have comparatively low insect susceptibility. The above findings may stimulate research towards the use of an insect susceptibility index as the screening method for low ODAP and trypsin inhibitor contents in lathyrus.

A sensitive color reaction of O-phthalaldehyde with DAPA was described by Rao (1978b) to determine ODAP in tissues of rats injected with the neurotoxin and also its content in the seeds. The results of the analysis was found to be comparable to those obtained by the ninhydrin procedure and by radioactivity. Rao (1978b) showed that the procedure is rapid and would be of use in studies with DAPA and ODAP.

MATERIALS AND METHODS

A. Preparation of the seed samples

The Lathyrus sativus Linn. samples used in these experiments were the cultivar "seminis albi" obtained from Mr. J. Furgal, (University of Manitoba) or the cultivar "NC8-8" obtained from Dr. M. Stauffer (Morden, Manitoba). The 1,000-kernel weight of the cultivar "seminis albi" was 270±2 g. For soyabean meal and wheat (or soy-wheat) diets, the utility cultivar "Glenlea" wheat was used. Heat-treated soyabean meal was obtained from Feed-Rite Ltd. (Winnipeg, Manitoba). Both the Lathyrus sativus Linn. var. seminis albi and the Glenlea wheat were harvested from plots at the Glenlea Agricultural Research Station, Manitoba by the Department of Plant Science of the University of Manitoba. The seeds were not cleaned prior to being incorporated into the diets. All seed samples were ground to pass through a 2 mm screen and were homogenously mixed using the facilities at the University's Poultry Shed or Feed Mill.

B. Formulation of diets

Generally all the diets were formulated to meet the minimum NRC recommendations (1977) for replacement pullets, unless a nutrient deficiency was tested. The diets were made isonitrogenous by varying the amount of protein supplement. Since metabolizable energy values of lathyrus have not been reported by NRC, Bulletin No. 1, the value (11.29 MJ/kg) reported in Latif et al. (1975) was used. An attempt was made to formulate all diets isocalorically. The energy levels in the diets were adjusted by adding either soyabean oil or corn starch to increase, or cellulose to decrease the caloric value. The dietary

ingredients were mixed by means of a Hobart 3-speed mixer. All diets were fed in mash form.

C. General bird management and experimental design

Day-old white Leghorn cockerels purchased from a commercial hatchery were housed in electrically heated, thermostatically controlled (Petersime) battery brooders with raised wire floors, and equipped with continuous lighting. From the time of arrival to the commencement of the experiments, the chicks were reared on commercial chick starter crumble diets containing a minimum of 21% crude protein. Prior to the initiation of each experiment the birds were fasted for 4 hr. Thereafter they were placed in weight groups from which they were randomly assigned to the experimental groups (replicates) which had the same mean weight. Feed and water were supplied ad libitum for all studies except for treatment G (pair-feeding) in Experiment III. The chicks were starved for 4 hr prior to weighing at the termination of each experiment. The difference between the initial and final weights of each pen of chicks was taken as the gain in body weight. The feed:gain ratio was calculated by dividing the feed intake (g) by the body weight gain (g). Other parameters measured are described in the appropriate experimental sections.

A completely randomized design involving different dietary treatments was employed in all experiments except in Experiment II where a randomized complete block design was carried out. The initial age of chicks prior to being put on test, the duration of the experiment and the average initial weight ± S.D. of the chicks are given in each experiment.

Treatment differences obtained from the statistical analysis of the data (Snedecor and Cochran, 1967) were subjected to the Student-Newman-Keuls multiple range test (Kirk, 1968).

D. Analyses

I. General analyses

Dry matter, ether extract, crude fibre, acid detergent fibre and ash were determined by methods according to the Association of Official Analytical Chemists (1975). Lignin content was determined according to the method of Goering and Van Soest (1970). Crude protein content was determined by the micro-Kjeldahl procedure which will be discussed later. Amino acid compositions of samples were determined according to the method of Spackman et al. (1958). Samples were ground and hydrolyzed with 6N HCl at 121°C for 16 hr. Amino acid analyses were carried out using a Beckman Model 119C, single column, amino acid analyzer. Cystine and methionine were determined according to the method in Hirs (1967). Mineral compositions of samples were determined using an atomic absorption spectrophotometer[1] according the the procedures in Analytical Methods for Atomic Absorption Spectrophotometry (1973) and Association of Official Analytical Chemists (1975). Selenium was determined by methods of Olson et al. (1975), and Whetter and Ullrey (1978). Chromic oxide in diets and fecal samples were determined by the method described in Williams et al. (1963).

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[1]Perkin-Elmer Absorption Spectrophotometer 303.

II. Micro-kjeldahl procedure for percent crude protein determination

The percent crude protein of samples were determined by the micro-kjeldahl procedure described in the Association of Official Analytical Chemists (1975) with partial modifications. The procedure was as follows:

1. Sample preparation

a) For solid samples: Fifty to one hundred milligrams of of sample was transferred into a 30 ml regular kjeldahl flask.

b) For liquid samples: Approximately 3 to 10 ml of the sample was pipetted into a 30 ml regular kjeldahl flask together with 50 mg of sucrose. Sucrose was utilized to ensure complete digestion of the sample.

2. Digestion of the samples

a) To each kjeldahl flask 1.9±0.1 g K_2SO_4, 40±10 mg $CuSO_4$ and 2.0±0.1 ml 95-98% H_2SO_4 was added.

b) The kjeldahl flask with sample was placed on a digestion rack heater[1] and heat control knob was turned to 3 for 15-20 min. The temperature of the control knob was then gently increased to 7, and the sample was mixed occasionally to avoid overshooting. Digestion required between 2-3 hr depending on the nitrogen content of the samples and the nature of the compounds being digested.

3. Distillation

a) When the digestion sample became clear, it was cooled and

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[1]LabCon Co. digestor model A, K.C., Mo.

5-10 ml of distilled water was added to dissolve the solids. The distillation apparatus[2] was turned on and the sample was poured into the distillator. The flask was washed 2-3 times with 2-3 ml of distilled water.

b) Five milliliters of 2% boric acid with 2-4 drops of indicator (methyl red - bromocresol green solution) in a 125 ml Phillip's beaker was connected to the distillator outlet.

c) Nine to ten milliliters of 45% NaOH was poured into distillator and 15 ml of distillate was collected.

4. Titration

a) A 50 ml burette was filled with standardized HCl (about 0.01N) and the distillate was titrated with constant stirring until the solution first became violet.

5. A blank analysis was also carried out.

6. The percent nitrogen in the sample was calculated by the following equation:

$$\% N = ((ml\ HCl - ml\ blank) \times normality\ of\ the\ HCl \times 14.007 \times 100) \div mg\ sample.$$

7. Percent nitrogen was converted to percent crude protein by multiplying the value by the factor, 6.25.

III. Percent soluble nitrogen analysis

The percent soluble nitrogen in the samples were determined by the following method:

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[2]LabCon Co. distillator, 8811 Prospect, K.C., Mo.

1. The amount of nitrogen in 1 g of original sample was determined by the micro-kjeldahl procedure (described in subsection DII).

2. The amount of soluble nitrogen in the original sample was determined by the following procedure:

a) One gram of original sample was homogenized for 0.5 min, using a Polytron homogenizer[1], with 20 ml of distilled water. The solution was rehomogenized after 30 min for 0.5 min, allowed to stand at room temperature (about 22°C) for 30 min and then centrifuged at 48,200xg for 20 min.

b) The amount of nitrogen in a 3-ml aliquot of the supernatant was determined by the micro-kjeldahl procedure (with the addition of 50 mg of sucrose).

c) The percent soluble nitrogen in the sample was calculated from the following equation:

$$\% \text{ soluble nitrogen} = \frac{N_s \times 20 \div 3}{N_o} \times 100\%$$

where N_s = amount of soluble nitrogen in the 3 ml aliquot (in mg).

N_o = amount of nitrogen in 1 g of the original sample (in mg)

IV. Determination of excreta pH

Samples of excreta were air dried for 3 days after the termination of the experiment and were stored at -20°C until analyzed.

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[1]Manufactured by Brinkmann, Ontario

Excreta samples (0.5 g) were transferred into Kimble centrifuge tubes (18.7 x 111 mm), 5 ml of distilled water (pH = 6.7) was added to each sample and they were homogenized with a Polytron homogenizer at top speed for 30 sec. After centrifuging at 27,000 x g for 15 min with a Sorvall centrifuge, a 3 ml aliquot of the supernatant was transferred into a small plastic beaker. The pH of the sample was determined using a Radiometer PHM62 Standard pH meter[1].

V. Determination of the amount of titrable acid in lathyrus after extraction at pH 5

Four 5-g replicate samples (lathyrus after extraction at pH 5) were added to 150 ml beakers and 100 ml of distilled water was added to each beaker. The samples were homogenized for 1 min using a Polytron homogenizer. The average pH reading was 5.27±0.04. The mixtures were then titrated with 0.2N NaOH to pH 6.55, and the amount of titrable acid was calculated.

VI. Determination of percent fat in dietary and fecal samples

Fat analysis was determined according to the procedure of Marchello, Dryden and Hale (1971) with minor modifications. The lipids were extracted from the dietary and fecal samples by the following procedure:

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[1]Distributed by Bach-Simpson Ltd., Ontario.

1. Five grams of finely ground sample was mixed with 100 ml of acid solvent consisting of 60:40:1 (V/V/V) of technical grade chloroform, methanol and hydrochloric acid for at least 13 min in an Ommi-mixer[1] at high speed.

2. The contents were filtered under vacuum through a plastic Buchner funnel containing Whatman No. 42 filter paper covered with a sheet of Whatman GF/A glass fiber filter paper.

3. Approximately 70 ml of filtrate was poured into a 100 ml graduated cylinder with a stopper, 20 ml of distilled water was added to the filtrate and the mixture was stirred vigorously.

4. The sample was allowed to extract overnight, after which 10 ml aliquots of the chloroform (lower) phase were pipetted into 50-ml Nalgene$^{(R)}$ containers.

5. The aliquots were dried under nitrogen gas for about 2 hr and then kept in a dessicator overnight (about 24 hr) before weighing.

6. A blank analysis was also carried out.

7. Percent fat in the sample was calculated from the following equation:

$$\% \text{ fat} = \left(\left(W_l \times \frac{V_{cp}}{10} \times \frac{100}{V_f} \right) - \text{blank} \right) \times \frac{100}{W_s}$$

Where W_l = wt of lipid in aliquot (in g)

V_{cp} = volume of chloroform (lower) phase (in ml)

V_f = volume of filtrate poured in graduated cylinder (in ml)

W_s = wt of sample (in g)

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[1]Model MM, manufactured by Lourdes Instrument Corp.

VII. Estimation of percent non-amino acid nitrogen in different lathyrus extracts

The percent non-amino acid nitrogen in different lathyrus extracts were calculated by subtracting the percent nitrogen equivalent of amino acids in different lathyrus extracts from their corresponding percent nitrogen values obtained by the micro-kjeldahl procedure.

E. Extraction methods

I. Acetone:water extraction of lathyrus

Several isolation procedures using different proportions of alcohol:water have been reported (Rao et al., 1964; Murti et al., 1964 and Nagarajan et al., 1965). Preliminary studies in our laboratory (unpublished) showed no difference in the amount of extracts obtained by either ethanol:water or acetone:water extractions. The degree of protein denaturation (judged by the amount of precipitate formed with 1:1 ratio of 10% sulphosalicylic acid and the aqueous extracts) was comparable between the two solvent extraction methods. Since the cost of feed:grade acetone is much cheaper than that of ethanol (95%), acetone:water extraction (50:50) was chosen and the following procedure employed:

1. One kilogram of ground lathyrus was extracted with 12 liters of a 50:50 mixture of distilled water and acetone using a Mechanical Mixer model 8A[1] for 15 min in a 20-liter plastic pail.

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[1]Manufactured by Ohmite Manufacturing Co., Ill., U.S.A.

2. The mixture was extracted at room temperature (about 22°C) for approximately 4 hr with occasionally mechanical mixing.

3. Thereafter, the mixture was filtered through a canvas bag. Pressure extraction was achieved by use of a mechanical-press[1].

4. The filtrate was distilled using a cyclone evaporator connected to a temperature controlled water pump Type FSE[2] at 80°C so as to remove excess acetone. The filtrate was distilled until no acetone smell was detected in the filtrate.

5. The concentrated filtrate was cooled and freeze dried[3].

6. The residue remaining after the filtering process (i.e. from step 3) was air dried using an electric fan for approximately 2 hr with frequent stirring to volatilize the acetone. The sample was then freeze dried.

7. The freeze dried samples of both the extract and the extracted lathyrus were stored at -20°C prior to diet preparation and amino acid analyses.

The total amount of acetone extracted lathyrus and acetone extract obtained after freeze drying represented about 82% of the original ground lathyrus sample. Of this amount, 10 and 90% of acetone extract and acetone extracted lathyrus were obtained respectively. The acetone extract was a dark brown, hygroscopic solid.

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[1]F. Dick - 9 litre press.

[2]Manufactured by Haake, Berlin-Steglitz, West Germany.

[3]All freeze drying was carried out using a temperature probe set at 45°C.

II. Water extraction of lathyrus with no pH alteration

1. Lathyrus was extracted by adding eight parts of boiling distilled water (by weight) to one part of ground lathyrus in a 20-liter plastic pail. The sample was mixed for 30 min using a Mechanical Mixer[1], followed by extraction for 4 hr at room temperature with occasional mixing (pH = 6.5).

2. The lathyrus-water mixture was centrifuged at 14,600 x g for 10 min after the 4-hr extraction period.

3. The supernatant was refrigerated and the sediment was collected and re-extracted by mixing four parts of hot distilled water (about 80°C) with one part of sediment for 2 hr at room temperature.

4. The mixture (pH = 6.5) was recentrifuged and the supernatant was pooled with the previous sample.

5. Both the freeze dried samples of the extracted lathyrus and the water extract were stored at -20°C before diet preparations and amino acid analyses.

The total amount of extracted lathyrus and water extract obtained after freeze drying represented about 84% of the original ground lathyrus sample. Of this amount, 27 and 73% of water extract and extracted lathyrus were obtained respectively. The water extract was a yellow, fluffy substance with a 94.2% dry matter content.

III. Water extraction of lathyrus following the adjustment of the pH of the mixture to approximately 5

The procedure was similar to that in subsection EII, except:

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[1]Same as that used in acetone:water extraction.

1. The first mixture was adjusted to pH 5.0±0.1 with the dropwise addition of 1 ml of 8N HCl for every 600 ml of the lathyrus-water mixture until a stable pH value of 5.0±0.1 was achieved. The preparation was mixed continuously.

2. During the re-extraction step (i.e. step 4 in subsection EII), the lathyrus-water mixture (with pH = 5.1) was adjusted to pH 5 using a total of 1 ml 8N HCl per 4 liters of mixture.

The amount of extract and extracted lathyrus obtained by this method was not determined. All the freeze dried samples were kept at $-20^{o}C$ prior to diet preparation and amino acid analyses.

F. Chick growth experiments

The following experiments involved administering different dietary treatments to growing white Leghorn chicks. Since significant responses in the criteria of performance were obtained when birds were on test for only a week, the duration of the experiments was restricted to periods of from 7 to 14 days except for Experiment II (28 days).

Experiment I. The effects of different levels of raw lathyrus on the growth of chicks.

The experimental design involved four treatments consisting of increasing levels of raw lathyrus replacing soyabean meal and wheat (Table 2) in six replicates; each replicate contained 8 birds. The experiment was initiated when the birds were seven days of age and terminated 7 days later. Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 7-day period.

Table 2. Formulas and analyses of diets (Experiment I)

Ingredients	Diet A	Diet B	Diet C	Diet D
	%	%	%	%
Lathyrus	-	25.00	50.00	75.00
Soyabean meal	28.25	19.20	9.15	-
Wheat[1]	62.00	46.00	31.00	15.10
Calcium carbonate	1.40	1.41	1.42	1.43
Calcium phosphate	1.45	1.49	1.53	1.57
Soyabean oil	5.00	5.00	5.00	5.00
Mineral mix[2]	0.50	0.50	0.50	0.50
Vitamin mix[3]	1.00	1.00	1.00	1.00
Chromic oxide	0.40	0.40	0.40	0.40
Chemical and calculated analyses of diets				
% Protein (Nx6.25)[4]	20.70	20.75	20.90	20.85
Metabolizable energy[5] (MJ/kg)	12.50	12.42	12.37	12.29

[1]DL-methionine was incorporated in the wheat fraction and the level of DL-methionine (% of diet) was as follows: diet A, 0.13; diets B and C, 0.15; and diet D, 0.17.

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

[3]The mineral mix (mg/kg of diet) was composed of: manganese, 129 as MnO; zinc, 11 as ZnO; iron, 7 as $FeSO_4.7H_2O$; copper, 6 as $CuSO_4.5H_2O$, and iodized NaCl, 4,757.

[4]Analyses represent the average dietary composition for each diet.

[5]Calculated values.

Experiment II. The effects on chicks of feeding lathyrus and soy-wheat diets for four one-week periods.

A randomized complete block design involved the use of two treatments (lathyrus and soy-wheat diets) and four one-week periods. There were six replicates per treatment and each replicate contained 8 birds. Formulas and analyses of diets are given in Table 3. The experiment was initiated when the birds were seven days of age and terminated 28 days later. Feed intake and weight gain were determined, and the corresponding feed: gain ratio calculated on days 7, 14, 21 and 28.

Experiment III. The effects of varying energy and protein levels, autoclaving and pair-feeding on the utilization of lathyrus and soy-wheat diets by the chicks.

The experimental design involved eight treatments consisting of diets with different energy and protein levels (Table 4). Autoclaving of the ground lathyrus, soyabean meal and wheat samples were carried out prior to the incorporation into the diets. The samples were spread in porcelain trays to a depth of 2 cm and autoclaved at 121^{o}C for 30 minutes. Pair-feeding was performed by providing an equivalent amount of feed to birds in group G to that consumed during the previous day by birds in group H. On the first day of the experimental period, feed was provided ad libitum. Each treatment consisted of six replicates; each replicate contained eight birds. The experiment was initiated when the birds were seven days of age and terminated ten days later. Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 10-day period.

Table 3. Formulas and analyses of diets (Experiment II)

Ingredients	Diet A	Diet B
	%	%
Lathyrus	-	81.7
Soyabean meal	31.5	-
Wheat	50.4	-
Calcium carbonate	1.2	1.2
Calcium phosphate	0.9	1.1
Other ingredients[1]	16.0	16.0
Chemical and calculated analyses of diets		
% Protein (Nx6.25)[2]	27.5	27.7
Metabolizable energy[3] (MJ/kg)	13.20	12.98

[1]The percent mineral mix, vitamin mix, chromic oxide, herring meal, amino acid mix and soyabean oil added to each diet was 0.5, 1.0, 0.4, 7.6, 0.5 and 6.0, respectively. The mineral and vitamin mixes were the same as those used in Experiment I. The composition of the amino acid mix (% of diet) was as follows: diet A, DL-methionine, 0.15; and corn starch, 0.35; diet B, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 0.18.

[2]Analyses represent the average dietary composition for each diet.

[3]Calculated values.

Table 4. Formulas and analyses of diets (Experiment III)

Ingredients	Diet A	Diet B	Diet C,E & G[1]	Diet D,F & H[1]
	%	%	%	%
Lathyrus	-	81.7	-	81.7
Soyabean meal	31.5	-	31.5	-
Wheat	50.4	-	50.4	-
Calcium carbonate	1.4	1.4	1.2	1.2
Calcium phosphate	1.5	1.7	0.9	1.1
Soybean oil	1.0	1.0	6.0	6.0
Corn starch	6.8	6.8	-	-
Cellulose[2]	5.0	5.0	-	-
Herring meal	-	-	7.6	7.6
Other ingredients[3]	2.4	2.4	2.4	2.4
Chemical and calculated analyses of diets				
% Protein (Nx6.25)[4]	21.3	21.0	27.1	27.2
Metabolizable energy[5] (MJ/kg)	10.93	10.71	12.77	12.56

[1]Grain samples used in diets C and D were fed raw while those in diets E and F were autoclaved at 121°C for 30 min prior to being incorporated into the diets. Diets G and H were the pair-fed raw diets.

[2]Alpha floc supplied by the Brown Company, Berlin, NH.

[3]The percent mineral mix, vitamin mix, chromic oxide and amino acid mix added to each diet was 0.5, 1.0, 0.4 and 0.5, respectively. The mineral and vitamin mixes were the same as those used in Experiment I. The composition of the amino acid mix (% of diet) was as follows: diets A, C, E and G, DL-methionine, 0.15; and corn starch, 0.35; diets B, D, F and H, DL-methionine, 0.22; L-tryptophan, 0.10 and corn starch, 0.18.

[4]Analyses represent the average dietary composition for each diet.

[5]Calculated values.

Experiment IV. The effects on chick performance of supplemental additions of one or more amino acids to a lathyrus-based diet.

A completely randomized design involving seven treatments with five replicates was employed. Each replicate contained six birds. Seven-day old chicks were used in this seven day feeding trial. The compositions of the control soy-wheat diet and the lathyrus diets are listed in Table 5. Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 7-day period.

Experiment V. Further studies on chick performance as affected by amino acid supplementation of a lathyrus-based diet.

A completely randomized design involving seven treatments with five replicates was employed. Each replicate contained five birds. The compositions of all the diets were the same as those in Experiment IV (Table 5, diet B), except the amino acid mix and percent protein formulated for each diet were different (Table 6). The experiment was initiated when the birds were seven days old and was terminated after seven days. Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 7-day period.

Experiment VI. The effects of autoclaving on the nutritional quality of lathyrus.

The experimental design involved six treatments consisting of lathyrus autoclaved at 121°C for 0 to 60 minutes, and a control soy-wheat diet. Autoclaving of the ground lathyrus was carried out prior to the incorporation into the diets. The samples were spread in porcelain trays to a depth of 2 cm and autoclaved at 121°C according to the following schedule: diet A, 0 min; diet B, 5 min; diet C, 15 min;

Table 5. Formulas and analyses of diets (Experiment IV)

Ingredients	Diet A	Diet B, C, D, E, F & G
	%	%
Lathyrus	-	82.00
Soyabean meal	31.00	-
Wheat	55.00	-
Calcium carbonate	1.40	1.15
Calcium phosphate	1.45	2.05
Soyabean oil	2.35	6.00
Amino acid mix[1]	6.90	6.90
Other ingredients[2]	1.90	1.90
Chemical and calculated analyses of diets		
% Protein (Nx6.25)[3]	20.2	(18.9 to 21.8)[4]
Metabolizable energy[5] (MJ/kg)	11.91	12.55

[1]Diets A to G consisted of different supplemental additions of amino acid(s) in the amino acid mix. The composition of the amino acid mix (% of diet) was as follows: diet A, DL-methionine, 0.15; and corn starch, 6.75; diet B, 0 amino acid; and corn starch, 6.9; diet C, DL-methionine, 0.25; and corn starch, 6.65; diet D, L-tryptophan, 0.15; and corn starch, 6.75; diet E, DL-methionine, 0.25; L-tryptophan, 0.15; and corn starch, 6.5; diet F, DL-methionine, 0.25; L-tryptophan, 0.15; L-glutamate, 4.0; and corn starch, 2.5; diet G, L-glutamate, 4.0; and corn starch, 2.9.

[2]The percent mineral mix, vitamin mix, and chromic oxide added to each diet was 0.5, 1.0 and 0.4, respectively. The mineral and vitamin mixes were the same as those used in Experiment I.

[3]Analyses represent the average dietary composition for each diet.

[4]The percent protein (Nx6.25) for diets B to G was as follows: diet B, 18.9; diet c, 19.5; diet D, 19.0, diet E, 19.2; diet F, 21.8; and diet G, 21.5.

[5]Calculated values.

Table 6. The compositions of the amino acid mixes and percent of protein of diets (Experiment V)

Ingredients	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F	Diet G
	%	%	%	%	%	%	%
DL-methionine	-	0.22	0.35	0.22	0.22	0.22	0.22
L-tryptophan	-	0.10	0.20	0.10	0.10	0.10	0.10
L-glutamate	-	-	-	2.00	4.00	6.0	-
L-aspartate	-	-	-	-	-	-	5.40
Anti-acid[1]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Corn starch	6.40	6.08	5.85	4.08	2.08	0.08	0.68
Protein[2]	19.2	19.4	19.7	20.7	21.9	23.2	22.9

[1]The composition of the anti-acid was as follows: 3 parts magnesium trisilicate ($Mg_2Si_3O_8$) and 1 part of aluminum-hydroxide ($Al(OH)_3.nH_2O$).

[2]Analyses represent the average dietary composition for each diet.

diet D, 30 min; and diet E, 60 min. The compositions of all the lathyrus diets (Diets A, B, C, D and E) were the same as those in Experiment III (Table 4, Diet D). The composition of the control soy-wheat diet was the same as those in Experiment III (Table 4, Diet C). The experiment was initiated when the birds were seven days old and terminated nine days later. Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 9-day period.

Experiment VII. The effects of a lyophilized acetone extract of lathyrus and acetone-extracted lathyrus on chick performance.

Acetone:water extraction was carried out according to the procedure in subsection EI. A completely randomized design involving five treatments with five replicates of four birds each was employed. The experiment was initiated when the birds were eight days old and was terminated after seven days. The formulas and analyses of the diets are given in Table 7. The percentage of the lyophilized lathyrus acetone extract added to the soy-wheat diet (Diet D) was in excess of that obtained upon acetone:water extraction (i.e. 11.4% vs 10.0%). Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 7-day period.

Experiment VIII. The effects on chick performance of a lyophilized water extract of lathyrus and water-extracted lathyrus.

Water extraction was carried out according to the procedure listed in subsection EII. A completely randomized design involving five treatments with five replicates of five birds each was employed. The experiment was initiated when the birds were eight days old and was terminated

Table 7. Formulas and analyses of diets (Experiment VII)

Ingredients	Diet A & B[1]	Diet C, D & E[2]
	%	%
Lathyrus	70.00	-
Soyabean meal	-	27.50
Wheat	-	40.00
Calcium carbonate	0.90	1.10
Calcium phosphate	1.55	1.10
Supplement mix[3]	9.65	9.65
Cellulose[4]	-	2.75
Other ingredients[5]	17.90	17.90
Chemical and calculated analyses of diets		
% Protein (Nx6.25)[6]	23.6 and 24.7	23.9, 24.3 and 24.1
Metabolizable energy[7] (MJ/kg)	12.95	12.77

[1]Lathyrus used in diet B was the freeze dried sample obtained after acetone:water extraction, while the lathyrus in diet A was raw lathyrus.

[2]Eight percent of the lathyrus acetone extract and amino acids equivalent to those contained in 8% of the lathyrus acetone extract were in the supplement mix of diets D and E, respectively. Diet C was the standard soy-wheat control diet. The average amino acid composition of the extract is given in Table 15.

[3]The composition of the supplement mix (% of diet) was as follows: diets A and B, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 9.33; diet C, DL-methionine, 0.15; and corn starch, 9.50; diet D, lathyrus acetone extract, 8.0; DL-methionine, 0.15; and corn starch, 1.50; diet E, DL-methionine, 0.16; L-lysine, 0.057; L-histidine, 0.014; L-arginine, 0.034; L-aspartate, 0.162; L-threonine, 0.026; L-serine, 0.021; L-glutamate, 0.139; L-proline, 0.015; L-glycine, 0.038; L-alanine, 0.026; L-cystine, 0.04; L-valine, 0.029; L-isoleucine, 0.013; L-leucine, 0.014; L-tyrosine, 0.007; L-phenylalanine, 0.014; and corn starch, 8.841.

[4]Same as in Experiment III.

[5]The percent mineral mix, vitamin mix, chromic oxide, herring meal and soyabean oil added to each diet was 0.5, 1.0, 0.4, 10.0 and 6.0, respectively. The mineral and vitamin mixes were the same as those used in Experiment I.

[6]Analyses represent the average dietary composition for each diet. Percent protein of each diet are listed individually.

[7]Calculated values.

after seven days. The formulas and analyses of the diets are given in Table 8. The percentage of the lyophilized lathyrus water extract added to the soy-wheat diet (Diet D) was in excess of that obtained upon water extraction (i.e. 30% vs 27%).

Parameters measured were feed intake, weight gain, excreta pH, apparent dry matter digestibility and percent crude protein retention. Percent dry matter, crude protein and the chromic oxide content of the dietary and fecal samples were determined by the procedures outlined in subsection D. The determination of excreta pH was carried out according to the procedure in subsection DIV. Percentages of the dry matter digested and the crude protein retained were calculated by the following equation:

$$\% \text{ nutrient digested/retained} = \frac{\%\ \text{nutrient in diet} - \left(\%\ \text{nutrient in feces} \times \frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in feces}}\right)}{\%\ \text{nutrient in diet}} \times 100\%$$

Experiment IX. Further studies of water-extractions, autoclaving, and the retention of nutrients by chicks fed different lathyrus and soy-wheat diets.

The procedures for the two methods of water extraction were outlined in subsection EII and EIII. Autoclaving of all the ground lathyrus, water-extracted lathyrus, soyabean and wheat samples was carried out prior to their incorporation into the diets. The samples were spread in porcelain trays to a depth of 2 cm and autoclaved at 121^{o}C for 30 minutes.

Table 8. Formulas and analyses of diets (Experiment VIII)

Ingredients	Diet A & B[1]	Diet C, D & E[2]
	%	%
Lathyrus	60.00	-
Soyabean meal	-	24.00
Wheat	-	36.25
Calcium carbonate	0.85	1.00
Calcium phosphate	1.75	1.35
Supplement mix[3]	19.50	19.50
Other ingredients[4]	17.90	17.90
Chemical and calculated analyses of diets		
% Protein (Nx6.25)[5]	22.5	(22.7, 28.5 and 26.4)[6]
Metabolizable energy[7] (MJ/kg)	13.33	13.47

[1]Lathyrus used in diet B was the freeze dried sample obtained after water extraction. Raw lathyrus was used in diet A.

[2]The supplement mix of diets D and E contained 18% lathyrus water extract and amino acids equivalent to those contained in 18% of the extract, respectively. Diet C was the standard soy-wheat control diet. The average amino acid composition of the extract is given in Table 15.

[3]The composition of the supplement mix (% of diet) was as follows: diets A and B, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 19.18; diet C, DL-methionine, 0.15; and corn starch, 19.35; diet D, lathyrus water extract, 18.0; DL-methionine, 0.15; and corn starch, 1.35; diet E, DL-methionine, 0.17; L-lysine, 0.302; L-histidine, 0.113; L-arginine, 0.360; L-aspartate, 0.529; L-threonine, 0.139; L-serine, 0.175; L-glutamate, 0.803; L-proline, 0.146; L-glycine, 0.169; L-alanine, 0.146; L-cystine, 0.083; L-valine, 0.180; L-isoleucine, 0.151; L-leucine, 0.234; L-tyrosine, 0.086; L-phenylalanine, 0.151; and corn starch, 15.563.

[4]Same as those used in Experiment VII.

[5]Analyses represent the average dietary composition for each diet.

[6]Percent protein of diets C, D and E is individually listed.

[7]Calculated values.

The experimental design involved eleven treatments in four replicates, each of which contained four birds. The experiment was conducted using eight-day old birds and lasted seven days. Two kinds of lyophilized lathyrus water extracts were added to diets containing soy-wheat as the cereal in order to assess the influence of these extracts on chick performance. Diets containing water-extracted lathyrus and autoclaved samples of lathyrus, water-extracted lathyrus, soyabean meal and wheat were also fed (Table 9).

Feed intake and weight gain were determined, and the corresponding feed:gain ratio calculated for the 7-day period. Other parameters measured in treatments A, B, C, D, E, G and H included percent nitrogen retention, apparent digestibility of dry matter, fat and amino acid(s). Percent dry matter, amino acid composition and the chromic oxide content of the dietary and fecal samples were determined according to the procedures outlined in subsection D. Percent fat in the samples were determined by the procedure described in subsection DVI. Percent nitrogen in the samples was obtained by converting the amino acids and ammonia in the dietary and fecal samples to their nitrogen equivalent values. Percent nitrogen retention and apparent digestibility of different nutrients were calculated by the equation listed in Experiment VIII.

Table 9. Formulas and analyses of diets (Experiment IX)

Ingredients	Diet[1] A and B	Diet[2] C and D	Diet[3] E,F,G,H,I and K	Diet[4] J
	%	%	%	%
Lathyrus	60.00	-	-	-
Water-extracted lathyrus	-	57.00	-	60.00
Soyabean meal	-	-	24.00	-
Wheat	-	-	28.00	-
Calcium carbonate	0.85	0.70	1.00	1.00
Calcium phosphate	1.75	2.05	1.50	2.20
Supplement mix[5]	19.50	22.35	23.50	24.60
Cellulose[6]	-	-	4.10	-
Herring meal	10.00	10.00	10.00	4.30
Other ingredients[7]	7.90	7.90	7.90	7.90
Chemical and calculated analyses of diets				
% Protein (Nx6.25)[8]	21.5	20.5	(20.5 to 25.5)	20.5
Metabolizable energy[9] (MJ/kg)	13.33	13.42	13.18	13.34

[1]Lathyrus used in diet B was autoclaved prior to diet preparation. Raw lathyrus was used in diet A.

[2]Lathyrus was the freeze dried sample obtained after water extraction with no pH alteration. Water-extracted lathyrus used in diet D was autoclaved prior to diet preparation.

[3]Diet E was the standard soy-wheat control diet. Grain samples of diet F were autoclaved prior to being incorporated into the diet. Eighteen percent lyophilized lathyrus water extracts, obtained from extraction methods in subsection EII and EIII, were incorporated in the supplement mix of diets G and I, respectively. Amino acids equivalent to 18% of the lathyrus water extract (with no pH alteration) were inserted in the supplement mix of diet H. Equivalent amount of titrable acid in lathyrus extracted at pH 5 (refer to the procedure outlined in subsection DV) was incorporated in the diet K.

[4]Lathyrus was the freeze dried sample obtained after water extraction (with pH adjustment).

Continued.....

Table 9 (Continued)

[5]The composition of the supplement mix (% of diet) was as follows: diets A and B, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 19.18; diets C and D, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 22.03; diets E, F and K, DL-methionine, 0.15; and corn starch, 23.35; diet G, lathyrus water extract (no pH alteration), 18; DL-methionine, 0.15; and corn starch, 5.35; diet H, amino acid composition was the same as that used in the supplement mix of diet E in Experiment VIII, 3.937; and corn starch, 19.563; diet I, lathyrus water extract (with pH adjusted to 5), 18; DL-methionine, 0.15; and corn starch, 5.35; diet J, DL-methionine, 0.22; L-tryptophan, 0.10; and corn starch, 24.28.

[6]Same as in Experiment III.

[7]The percent mineral mix, vitamin mix, chromic oxide and soyabean oil added to each diet was 0.5, 1.0, 0.4 and 6.0, respectively. The mineral and vitamin mixes were the same as those used in Experiment I.

[8]Analyses represent the average dietary composition for each diet. Percent protein for each diet was as follows: diet A and B, 21.5; diet C and D, 20.5; diet E, 21.0; diet F, 20.5; diet G, 25.5; diet H, 25.2; diet I, 23.2; diet J and K, 20.5.

[9]Calculated values.

RESULTS AND DISCUSSION

A. Composition of Lathyrus sativus Linn.

The objective of this study was to establish the gross composition of different lathyrus fractions as only limited information is currently available in the literature. The 1,000-kernel weight of lathyrus was approximately 270 g which agrees with the value given by Furgal (personal communication). The ratio of cotyledon:testa was 9 to 1. The gross chemical composition of different fractions of lathyrus is given in Table 10. Results from the present study on the composition of the whole seed fall within the limits reported by other researchers (Malik et al., 1967, Sarma and Padmanaban, 1969; Panda et al., 1972 and Latif et al., 1975). Analyses of the testa and cotyledon have not been previously reported. Crude protein, calcium and phosphorus are mainly concentrated in the cotyledon fraction, whereas most of the crude fibre in lathyrus is located in the testa fraction.

Amino acid analyses of these fractions (Table 11) revealed similarities in the percentage of individual amino acid per total amino acids among the three fractions, particularly between the whole pea and cotyledon portion of the seed. The results of amino acid analyses showed that lathyrus relative to the requirements of the chick (NRC, 1977) is rich in lysine and contains relatively adequate concentrations of other essential amino acids except methionine and cystine. Tryptophan content in lathyrus was not determined in the present investigation.

The amino acid composition of two cultivars of lathyrus (Table 12) grown in Manitoba was also established. No significant difference was

Table 10. Gross chemical compositions of different fractions of Lathyrus sativus Linn. var. seminis albi

Nutrient (%, air dried basis)	Fraction[a]		
	Whole pea	Cotyledon	Testa
Nitrogen (N)	3.9 ±0.0	4.4 ±0.0	1.0 ±0.0
Protein (Nx6.25)	24.5 ±0.2	27.3 ±0.1	5.9 ±0.1
Dry matter	91.1 ±0.1	90.9 ±0.1	93.2 ±0.1
Fat (ether extract)	0.7 ±0.1	0.8 ±0.2	0.4 ±0.0
Crude fibre	5.4 ±0.1	1.1 ±0.1	43.6 ±0.4
Ash	2.6 ±0.1	2.6 ±0.0	2.8 ±0.1
Acid detergent fibre	7.6 ±0.3	2.0 ±0.0	56.6 ±0.1
Permanganate lignin	0.7 ±0.1	0.3 ±0.0	3.2 ±0.1
Cellulose	7.2 ±0.4	1.9 ±0.0	53.2 ±0.4
Gross energy (MJ/kg)	16.1 ±0.0	-	-
Calcium	0.14±0.00	0.09±0.00	0.61±0.00
Phosphorus	0.31±0.00	0.34±0.00	0.06±0.00

[a]Average values ± S.D. were from duplicate analyses.

Table 11. Comparison of the amino acid composition of different fractions of Lathyrus sativus Linn. var. seminis albi

Amino acid	Fraction[a]					
	Whole pea		Cotyledon[b]		Testa[b]	
	% of sample	% of total amino acid	% of sample	% of total amino acid	% of sample	% of total amino acid
Lysine	1.65	7.2	1.85	7.4	0.42	7.7
Histidine	0.64	2.8	0.69	2.8	0.13	2.3
Arginine	1.97	8.6	2.29	9.2	0.34	6.2
Aspartic acid	2.89	12.6	2.92	11.7	0.83	15.0
Threonine	1.00	4.4	1.09	4.4	0.28	5.2
Serine	1.16	5.1	1.22	4.9	0.33	5.9
Glutamic acid	4.27	18.6	4.41	17.7	0.72	13.0
Proline	0.98	4.3	1.06	4.3	0.24	4.3
Glycine	1.03	4.5	1.15	4.5	0.29	5.2
Alanine	1.11	4.8	1.22	4.9	0.31	5.7
Cystine	0.34	1.5	0.38	1.5	0.10	1.9
Valine	1.20	5.2	1.35	5.4	0.29	5.3
Methionine	0.20	0.9	0.27	1.1	0.07	1.2
Isoleucine	1.08	4.7	1.23	4.9	0.27	4.9
Leucine	1.69	7.4	1.90	7.6	0.42	7.7
Tyrosine	0.60	2.6	0.68	2.7	0.18	3.2
Phenylalanine	1.10	4.8	1.25	5.0	0.29	5.3
Total	22.91	100.0	24.96	100.0	5.51	100.0

[a]Average values were from duplicate analyses. The crude protein content is given in Table 10.

[b]The ratio of cotyledon:testa was 9:1 on an air dried basis.

Table 12. Comparison of the amino acid composition of two cultivars of Lathyrus sativus Linn.

Amino acid	Cultivar[a]			
	seminis albi[b]		NC8-8[b]	
	% of sample	% of total amino acids	% of sample	% of total amino acids
Lysine	1.65	7.2	1.75	7.7
Histidine	0.64	2.8	0.66	2.9
Arginine	1.97	8.6	1.94	8.5
Aspartic acid	2.89	12.6	2.74	12.0
Threonine	1.00	4.4	0.89	3.9
Serine	1.16	5.1	1.15	5.1
Glutamic acid	4.27	18.6	4.04	17.8
Proline	0.98	4.3	1.06	4.6
Glycine	1.03	4.5	0.98	4.3
Alanine	1.11	4.8	1.04	4.6
Cystine	0.34	1.5	0.41	1.8
Valine	1.20	5.2	1.25	5.5
Methionine	0.20	0.9	0.19	0.9
Isoleucine	1.08	4.7	1.10	4.9
Leucine	1.69	7.4	1.74	7.6
Tyrosine	0.60	2.6	0.58	2.6
Phenylalanine	1.10	4.8	1.20	5.3
Total	22.91	100.0	22.72	100.0

[a]Average values were from duplicate analyses.

[b]The crude protein content of seminis albi and NC8-8 was estimated to be 24.53 and 25.62% (Nx6.25), respectively.

noted between the creamy-white seeded cultivar "seminis albi" and the mottled cultivar "NC8-8". However different amino acid values as indicated in Table 1 were reported by Sarma and Padmanaban (1969) and Latif et al. (1975). Results from the present study revealed lower histidine and tyrosine values than those reported by the previous two researchers. The concentrations of lysine, serine, proline, cystine and methionine of the two cultivars that were analyzed in the current study were intermediate to the levels reported by Sarma and Padmanaban (1969) and Latif et al. (1975). However, higher values were obtained in the current study relative to those of the previous authors for the remaining amino acids. The variation in the findings of Sarma and Padmanaban (1969), Latif et al. (1975) and the present investigation might be caused by different analytical procedures and/or possibly to the use of different cultivars.

The average mineral composition of the two cultivars (Table 13) revealed varying degrees of differences among all minerals. The element which showed the greatest difference in composition when expressed as the percentage of the lower value between the two cultivars was selenium (110%), followed by managanese, iron, sodium, and zinc; that showing the least difference was potassium (2%), followed by phosphorus, copper, magnesium and calcium. These differences are attributable to the use of different cultivars and/or environmental variation. The concentrations of phosphorus, magnesium and manganese however are within the range of values cited in the literature review section. The calcium content in lathyrus obtained from the present study was significantly lower than the range of reported values (i.e. 0.14% versus 0.22 to 0.28%). Moreover,

Table 13. Mineral compositions of two cultivars of Lathyrus sativus Linn.

Element	Cultivar[a] Seminis albi	NC8-8
Calcium	0.141±0.002%	0.109±0.005%
Phosphorus	0.318±0.001%	0.343±0.001%
Iron	64.2±1.0 ppm	115.0±3.6 ppm
Copper	6.3±0.3 ppm	7.2±0.3 ppm
Magnesium	0.143±0.002%	0.119±0.003%
Manganese	11.1±0.4 ppm	21.3±2.6 ppm
Zinc	31.8±2.5 ppm	41.5±0.7 ppm
Sodium	330.5±24.7 ppm	204.0±5.7 ppm
Potassium	1.103±0.068%	1.120±0.029%
Selenium	0.10±0.02 ppm	0.21±0.04 ppm

[a]Average values ± S.D. were from 4 replicate analyses.

the selenium content in the two cultivars analysed in the current study was between 0.1 to 0.2 ppm which is far below the toxic level (229 ppm) reported by Rudra (1952). The seleniferous ability of lathyrus is not known. It might be possible that selenium toxicity by lathyrus is endemic rather than pandemic. Further agronomical studies are needed to substantiate the possible seleniferous nature of lathyrus.

B. Composition of extracted lathyrus and lathyrus extract after different extraction methods

The objectives of this study were to establish the amino acid and non-amino acid nitrogen content, as a possible index of the level of antinutritional factors, in various lathyrus extracts. The different extracts and corresponding extracted fractions of lathyrus that were obtained in this portion of the study were subsequently fed to chicks to determine if the antinutritional or growth inhibitory activity of lathyrus was concentrated in a particular fraction.

The amino acid composition of lathyrus (i.e. concentration of individual amino acids relative to the total amino acid concentration) was not affected by the extraction procedures (Table 11 and 14). The crude protein content was also similar between the water-extracted lathyrus (no pH adjustment) (24.47%) and untreated lathyrus (24.53%). There was however an increase in the crude protein content of the acetone:water extracted lathyrus (25.94%) and the water-extracted lathyrus with acidic solution (29.75%). These increases may be attributed to the decrease in extractability of protein and non-protein nitrogen from lathyrus in either an acetone solution or an acidic medium. Wolf and Cowan (1971) have also reported a decrease in the protein solubility

Table 14. Amino acid compositions of Lathyrus sativus Linn. var. seminis albi after different extraction methods.

Amino acid	Fraction[a]					
	Acetone:water-extracted lathyrus[b]		Water-extracted lathyrus (no pH adjustment)[b]		Water-extracted lathyrus (pH adjusted to 5)[b]	
	% of sample	% of total amino acids	% of sample	% of total amino acids	% of sample	% of total amino acids
Lysine	1.81	7.6	1.78	7.7	2.14	7.5
Histidine	0.71	3.0	0.68	3.0	0.86	3.0
Arginine	2.03	8.6	1.88	8.2	2.49	8.8
Aspartic acid	2.86	12.1	2.73	11.9	3.32	11.6
Threonine	0.96	4.1	0.96	4.2	1.11	3.9
Serine	1.20	5.1	1.20	5.2	1.46	5.1
Glutamic acid	4.21	17.8	3.83	16.6	5.01	17.6
Proline	0.97	4.1	0.98	4.3	1.38	4.8
Glycine	1.03	4.4	1.01	4.4	1.24	4.4
Alanine	1.08	4.6	1.08	4.7	1.26	4.4
Cystine	0.41	1.7	0.37	1.6	0.44	1.5
Valine	1.31	5.5	1.33	5.8	1.59	5.6
Methionine	0.22	0.9	0.21	0.9	0.25	0.9
Isoleucine	1.18	5.0	1.20	5.2	1.45	5.1
Leucine	1.84	7.8	1.87	8.1	2.27	8.0
Tyrosine	0.54	2.3	0.59	2.6	0.69	2.4
Phenylalanine	1.28	5.4	1.30	5.6	1.55	5.4
Total	23.64	100.0	23.00	100.0	28.51	100.0

[a]Average values were from duplicate analyses.

[b]The crude protein content of acetone:water-extracted lathyrus, water-extracted lathyrus (no pH adjustment), and the other water-extracted lathyrus was estimated to be 25.94, 24.47 and 29.75% (Nx6.25), respectively.

of soybeans under acidic conditions (i.e. between pH 4.2 to 4.6).

Analyses for amino acids in the different lathyrus extracts (Table 15) show differences in amino acid composition when individual values were expressed either as a percentage of the sample or as a percentage of total amino acids. The amino acid composition of the lathyrus acetone extract and water extract (pH adjusted to 5) when expressed as a percentage of its air dried weight was similar. Also, most of the essential amino acids except cystine, in these two extracts were lower in concentration than those in the lathyrus water extract (no pH adjustment). However a different observation was obtained when individual amino acids were expressed as a percentage relative to its total amino acid concentration. In such instances, only histidine, arginine, serine, proline, isoleucine, leucine, tyrosine and phenylalanine in the acetone extract and water extract (pH adjusted to 5) were lower than those in the water extract (no pH adjustment); whereas the levels of lysine, aspartic acid, glycine, alanine, cystine and methionine were slightly higher in the previous two extracts than those in the one with no pH adjustment. The levels of threonine, glutamic acid and valine were similar in all extracts. The difference in amino acid composition may be in part attributed to the decrease in protein solubility in an acidic medium or in acetone.

The relative amount of acetone extract and water extract (no pH adjustment) isolated from 100 g of lathyrus was 8 and 23 g, respectively. The relative amount of water extract isolated with acidic solution however was not established. The data (Table 16) show that the amount of non-amino acid nitrogen per unit dry weight of extract was the highest

Table 15. Amino acid compositions of different kinds of Lathyrus sativus Linn. var. seminis albi extracts

Amino acid	Fraction[a]					
	Lathyrus acetone extract[b]		Lathyrus water extract[b] (no pH adjustment)		Lathyrus water extract[b] (pH adjusted to 5)	
	% of sample	% of total amino acids	% of sample	% of total amino acids	% of sample	% of total amino acids
Lysine	0.71	8.7	1.68	8.0	0.77	11.4
Histidine	0.18	2.2	0.63	3.0	0.15	2.2
Arginine	0.42	5.1	2.00	9.5	0.41	6.0
Aspartic acid	2.02	24.7	2.94	14.0	1.35	19.9
Threonine	0.32	3.9	0.77	3.7	0.30	4.4
Serine	0.26	3.2	0.97	4.6	0.25	3.6
Glutamic acid	1.74	21.3	4.46	21.1	1.46	21.5
Proline	0.19	2.3	0.81	3.8	0.15	2.1
Glycine	0.48	5.9	0.94	4.5	0.39	5.7
Alanine	0.32	3.9	0.81	3.8	0.36	5.3
Cystine	0.50	6.2	0.46	2.2	0.50	7.4
Valine	0.36	4.4	1.00	4.8	0.28	4.2
Methionine	0.06	0.8	0.11	0.5	0.06	1.0
Isoleucine	0.16	1.9	0.84	4.0	0.13	2.0
Leucine	0.18	2.2	1.30	6.2	0.16	2.4
Tyrosine	0.09	1.1	0.48	2.3	Trace	-
Phenylalanine	0.18	2.2	0.84	4.0	0.06	0.9
Total	8.17	100.0	21.04	100.0	6.78	100.0

[a]Average values were from duplicate analyses.

[b]The crude protein content of lathyrus acetone extract, lathyrus water extract (no pH adjustment), and the other lathyrus water extract was estimated to be 22.53, 28.96 and 16.03% (Nx6.25), respectively. The amount of extract isolated from 100 g of lathyrus was 8 g and 23 g for the lathyrus acetone extract and lathyrus water extract (no pH adjustment), respectively. The amount of water extract isolated under acidic condition was not determined.

Table 16. Percent non-amino acid nitrogen in the different lathyrus extracts

Lathyrus extract	(a) Total nitrogen[1] (%)	(b) Amino acid nitrogen[2] (%)	(c) Non-amino acid nitrogen[3] (%)
Acetone extract	3.61	1.10[4]	2.51[5]
Water extract (no pH adjustment)	4.63	3.01[4]	1.62[5]
Water extract (pH adjusted to 5)	2.57	0.95	1.62

[1]Percentage was estimated by the micro-kjeldahl procedure.

[2]Percentage was calculated from the nitrogen content of each amino acid and the corresponding amino acid composition of each extract (Table 15).

[3]c = (a-b), except the two isomers of the neurotoxin, ODAP, and all the other amino acids that were not determined in the amino acid analysis.

[4]The percent amino acid nitrogen isolated from 100 g of lathyrus from acetone:water extraction and water extraction under neutral condition was 0.09 and 0.69%, respectively.

[5]The percent non-amino acid nitrogen isolated from 100 g of lathyrus from acetone:water extraction and water extraction under neutral condition was 0.20 and 0.37%, respectively.

in the acetone extract and was similar in the two water extracts. These data would suggest that if the antinutritional factor(s) was concentrated in the non-amino acid fraction then the greatest depression in growth on a per unit of dry weight basis should occur when chicks were fed the acetone extract as compared to that obtained with equal amounts of either of the other two water extracts. However, the percent non-amino acid nitrogen isolated from 100 g of lathyrus with the water extraction (no pH adjustment) procedure was higher than that of the acetone:water procedure (i.e. 0.37 versus 0.20%). This effect may be attributed to the decrease in solubility of the non-amino acid nitrogen in acetone. Overall it may be concluded that water extraction is a more efficient procedure than the (50:50) acetone:water fractionation in the isolation of non-amino acid nitrogen from lathyrus but that the acetone: water fractionation yields a more concentrated form of non-amino acid nitrogen in per unit dry weight basis of the lyophilized extract.

The amino acid nitrogen content in the lathyrus water extract (no pH adjustment) was higher than that of the acetone extract, when either expressed as a percentage per unit dry weight of the extract (i.e. 3.01 versus 1.10%) or as a percentage isolated from 100 g of lathyrus (i.e. 0.69 versus 0.09%). These observations would imply that the water extract (no pH adjustment) contains higher absolute levels of proteins and also higher levels of potential antinutritional factors that are protein in nature (i.e. trypsin inhibitors and phytohemagglutinins) rather than the acetone extract. Roy (1972b) and Latif _et al_. (1975) have reported the presence of trypsin inhibitors and phytohemagglutinins in lathyrus. Since the relative amount of lathyrus water extract isolated

with acidic medium was not determined, no definite conclusion could be made regarding this extract. However, the data in Table 16 show that the total nitrogen and the amino acid nitrogen content of the acidic water extract was the lowest of the three extracts. These observations would suggest a lower concentration of possibly protein-containing inhibitors in the acidic extract. It may be concluded that water extraction under neutral condition is the most efficient of the three extraction methods for the isolation of possible protein-containing inhibitors (i.e. trypsin inhibitors and phytohemagglutinins) and also the non-amino acid nitrogen compounds[1] from lathyrus. Results from the feeding experiments of these extracts and the corresponding extracted fractions will be discussed in the later subsections.

C. Chick growth experiments

Experiment I

The performance data in this experiment and all subsequent experiments are presented on an average chick basis.

The objective of this study was to examine the feed intake, body weight gain and feed:gain ratio of young Leghorn cockerels fed diets containing varying levels of lathyrus. The results (Table 17) show that feed consumption, weight gain and FCE by chicks were decreased with increasing levels of lathyrus in the diets. The depressions ($P<0.05$) in weight gain for chicks fed diets containing 25, 50 and 75% lathyrus as compared to those fed the soy-wheat diet (or 0% lathyrus) were 9, 23 and 40%

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[1] Refer to Table 16, footnote 3.

Table 17. The performance of chicks fed different levels of raw lathyrus. (Experiment I)

Treatment[a]	Response criterion[b]		
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)
Lathyrus, 0% (A)	116.2^{bc}	65.3^{D}	1.78^{A}
Lathyrus, 25% (B)	119.1^{c}	59.3^{C}	2.01^{B}
Lathyrus, 50% (C)	112.2^{b}	50.3^{B}	2.24^{C}
Lathyrus, 75% (D)	98.7^{a}	39.2^{A}	2.54^{D}
Standard error	1.9	1.4	0.04

[a]Letters in brackets refer to diet designations in Table 2.

[b]The initial average weight of the 7-day old chicks was 73.0±0.3 g. The duration of the experiment was 7 days. Means within each column not sharing a common non-capitalized or capitalized superscript are significantly different at $P<0.05$ or $P<0.01$, respectively.

respectively. Increased growth depressions in animals fed diets containing increased levels of lathyrus have been reported (Bhagvat, 1946; Sastry et al., 1963 and Malik et al., 1967). In addition, Malik et al. (1967) reported significant ($P<0.05$) impairments in feed consumption and feed efficiency of broiler chicks fed a diet containing 25% of lathyrus. Latif et al. (1975) also showed that chicks fed on lathyrus diets grew more slowly than those on the maize-soya control diet but that higher percentages of lathyrus (i.e. 22 and 37%) did not depress growth rate any more than the 10 per cent level. Moreover, Latif et al. (1975) reported significant ($P<0.05$) decreases in feed consumption with increased amounts of lathyrus in broiler chick diets which agrees with the present findings.

No symptom of experimental neurolathyrism (i.e. paralysis of the legs) was observed in the present study and in the reports of Malik et al. (1967) and Latif et al. (1975). However, Panda et al. (1972) reported that day-old unsexed Leghorn chicks fed on diets containing 25 or 50% lathyrus developed paralytic symptoms and all the chicks fed the 50% level died in two weeks. He proposed that death might be due to damage to the nuclei in the medulla oblongata that regulate heart beat and respiration or to the inability of the affected chicks to eat and drink. In the current study, chicks fed on lathyrus-containing diets seemed to be more hyperactive than those on the soy-wheat diet. There was however no conclusive evidence for the hyperirritability of lathyrus fed birds and no histological examination was performed in the present investigation. The difference between the present findings and those of Panda et al. (1972) may be due to the use of different varieties of lathyrus, and the

difference in the age of birds when first fed the lathyrus containing diets. In the experiments reported by Malik et al. (1967), Panda et al. (1972) and Latif et al. (1975), the birds were placed on test at one day of age whereas in the current experiment birds were placed on test when they were seven days old. Due to the possible early development of the BBB in chicks, the susceptibility of the lathyrus neurotoxin(s) in the 7-day old chicks might be lesser than that of the day-old chicks. Rao and Sarma (1967) were able to show that ODAP, the principle causative substance responsible for neurolathyrism, did not induce any neurotoxic symptoms in normal adult birds at doses that caused neurological disorders in day-old chicks. However, no information is available regarding the rate of development of the BBB in older chicks. On the other hand, the entry of the neurotoxin ODAP in the CNS of adult rat and normal monkeys with mature BBB was reported by different workers (Mehta et al., 1976 and 1980, Rao, 1978a, Parker et al., 1979) which was different from the findings of Rao and Sarma (1967). Further studies with the combination of nutritional and pharmacokinetic aspects of lathyrus are needed to establish the relationship of BBB development and experimental lathyrism in chicks.

Experiment II

The objective of this experiment was to study the feeding patterns of chicks fed a lathyrus and a control soy-wheat diet during a 4-week period. In the previous study it was shown that the severity of growth depression increased when birds were given higher levels of lathyrus in the diet. Therefore in this study a diet containing 82% lathyrus as

the sole grain source was used. The analysis of variance data (Table 18, 19) show that there were main effects ($P<0.01$) for both diet and period of treatment for all three criteria of performance. The average feed intake, weight gain and feed:gain ratio for birds fed the soy-wheat and lathyrus diets were 193 g and 164 g, 97 g and 63 g, and 1.94 and 2.55, respectively. The corresponding values for the diet x period interaction ($P<0.01$) for body weight gain are given in Table 18. The relative increase in weight gain from period one to period four for the lathyrus fed birds was 196% whereas the corresponding improvements for the soy-wheat fed birds was only 97%. These results demonstrate that the relative rates of growth of the lathyrus fed birds were progressively increased during the four-week period so that at the end of the test period lathyrus fed birds had weight gains that approached those for the soy-wheat fed birds. These findings suggest that there are growth inhibitory factors in lathyrus and that adaptation seems to occur. An adaptative mechanism has also been suggested by Adhya et al. (1975) who observed overall stimulation of the hemopoietic system of albino rats after parenteral administration of lathyrus neurotoxins. Additional studies should be carried out to more clearly establish the degree and nature of the adaptation.

Experiment III

The objectives of this experiment were to examine the effects of varying energy and protein levels, autoclaving, and pair-feeding of lathyrus- and soy-wheat-containing diets on the performance of the chicks. The data (Table 20) indicate that significant ($P<0.01$) improve-

Table 18. The performance of chicks fed raw lathyrus and soy-wheat diets for four one-week periods. (Experiment II)

Treatment		Response criterion[c]		
Diet[a]	Period[b]	Feed intake	Weight gain	Feed:gain
		(g)	(g)	(g/g)
Soy-wheat (A)	1	101.8^{B}	62.5^{B}	1.63^{A}
Lathyrus (B)	1	71.7^{A}	31.0^{A}	2.32^{B}
Soy-wheat (A)	2	157.9^{B}	89.5^{B}	1.77^{A}
Lathyrus (B)	2	135.5^{A}	55.7^{A}	2.44^{B}
Soy-wheat (A)	3	229.4^{B}	113.4^{B}	2.03^{A}
Lathyrus (B)	3	189.4^{A}	72.2^{A}	2.64^{B}
Soy-wheat (A)	4	284.6^{B}	123.0^{B}	2.31^{A}
Lathyrus (B)	4	258.5^{A}	91.7^{A}	2.81^{B}
Standard error		4.2	1.3	0.04

[a] Letters in brackets refer to diet designations in Table 3.

[b] Seven days duration for the four consecutive periods.

[c] The initial average weight of the 7-day old chicks was 56.7 ± 0.4 g. The duration of the experiment was four one-week periods (i.e. 28 days). Means within each column for the same period not sharing a common superscript are significantly ($P<0.01$) different.

Table 19. Summary of analysis of variance (Experiment II)

		Mean square		
Source of variation	df	Feed intake	Weight gain	Feed:gain
Diet	1	10,546**	14,241**	4.63**
Period	3	76,118**	8,297**	0.80**
Diet x period	3	174	65**	0.02
Experimental error	40	104	10	0.01

**Significant at P<0.01.

Table 20. The performance of chicks fed lathyrus and soy-wheat diets of varying energy and protein levels (Experiment III)

Treatment[a]	Response criterion[b]		
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)
Soy-wheat, low (A)	170.5^{c}	79.2^{d}	2.16^{B}
Lathyrus, low (B)	127.4^{ab}	38.5^{a}	3.32^{D}
Soy-wheat, high (C)	160.9^{c}	94.4^{e}	1.71^{A}
Lathyrus, high (D)	122.5^{ab}	49.3^{ab}	2.52^{C}
Autoclaved soy-wheat, high (E)	166.7^{c}	97.2^{e}	1.72^{A}
Autoclaved lathyrus, high (F)	145.1^{bc}	70.7^{cd}	2.06^{B}
Pair-feeding soy-wheat, high (G)	107.8^{a}	61.1^{bc}	1.77^{A}
Pair-feeding lathyrus, high (H)	115.0^{ab}	47.5^{ab}	2.44^{C}
Standard error	7.7	4.2	0.05

[a]Letters in brackets refer to diet designations in Table 4. "Low" and "high" terms designate diets that contain low and high energy and protein levels.

[b]The initial average weight of the 7-day old chicks was 63.4±0.3 g. The duration of the experiment was 10 days. Means within each column not sharing a common non-capitalized or capitalized superscript are significantly different at $P<0.05$ or $P<0.01$, respectively.

ments in FCE were observed in chicks fed both the lathyrus and soy-wheat diets that had higher protein (21% versus 27%) and energy (10.87 versus 12.54 MJ/kg) values. When the first four treatments were reanalyzed as a 2(soy-wheat versus lathyrus) x 2(low versus high energy and protein) factorial arrangement there was a significant ($P<0.01$) interaction in FCE between the two factors. However, the relative increase of the FCE values from low to high energy and protein levels for the soy-wheat- and lathyrus-fed chicks were only 21 and 24%, respectively. This significant interaction was attributed to both the relative decrease in feed consumption (6% versus 4%) and the relative increase in body weight gain (19% versus 28%) for the soy-wheat- and lathyrus-fed birds, respectively.

Results from autoclaved treatment of the lathyrus and the soy-wheat based diets show similar patterns to that of varying energy and protein levels, but the relative increase in the FCE values from non-autoclaved to autoclaved treatment for the soy-wheat and lathyrus-fed chicks (1% versus 18%) was much greater. There were also relative increases in feed intake for the chicks fed both the autoclaved soy-wheat and the autoclaved lathyrus diets of 4% and 18%, respectively. Moreover, there was a significant ($P<0.05$) increase in body weight gain in chicks fed the autoclaved lathyrus diet (43%) but not in those fed the autoclaved soy-wheat-containing diet. These results would suggest that heat treatment of lathyrus at 121^{o}C at 30 min denatures or destroys a compound(s) that reduces nutrient utilization by the animal and thereby reduces growth rate. The same effect, in contrast, does not occur when chicks are fed the autoclaved soy-wheat diet. The results of the present experiment were different from those reported by Malik et al. (1967) but generally

similar to those of Latif et al. (1975). Malik et al. (1967) reported no significant ($P>0.05$) difference in both feed consumption, body weight gain and FCE in broiler chicks fed the autoclaved and raw lathyrus (25%) diets. There was nevertheless a significant ($P<0.05$) improvement in the dressed weight of birds fed an autoclaved lathyrus diet for the eight-week period. In contrast, Latif et al. (1975) reported that autoclaved lathyrus fed birds showed significant ($P<0.05$) increases in feed consumption and body weight gain as compared to those fed on a raw lathyrus diet by an average of 25% and 33% from the two experiments. Moreover, these improvements resulted in growth rates of autoclaved fed birds similar to that obtained with the maize-soya control group, and FCE in the group fed an autoclaved lathyrus containing diet was significantly ($P<0.05$) better than that of the control group by 7% (Latif et al., 1975). In the present study, feed consumption, body weight gain and FCE were both lower in chicks fed an autoclaved lathyrus diet as compared to those fed a raw soy-wheat diet with the same levels of energy and protein. However, the performance of the birds on the high energy and protein autoclaved lathyrus diet were comparable to those fed the low energy and protein soy-wheat diet.

The results from the pair-feeding of lathyrus and soy-wheat diets in the present investigation demonstrate that there was no difference between birds fed the two diets in feed intake ($P>0.05$) and weight gain ($P>0.05$) but a significant difference ($P<0.01$) in FCE. The failure to achieve significant differences in weight gain between the lathyrus and soy-wheat fed birds may be in part attributed to the slightly lower feed intake in the soy-wheat fed birds. The results nevertheless

demonstrate that when feed intake levels were similar, FCE and possibly weight gain were lower in lathyrus fed birds as compared to those fed on the soy-wheat based diet. These findings therefore suggest that the poor performance with the lathyrus fed birds cannot be attributed solely to an appetite or palatability effect.

Experiment IV

This experiment was conducted to establish the limiting amino acid(s) in lathyrus. The data (Table 21) show significant ($P<0.01$) improvements in the performance of chicks fed diets supplemented with methionine with or without the combinations of tryptophan and/or glutamic acid, but the performance was still inferior to those on the soy-wheat control diet. Chicks fed a diet with no amino acid supplementation and diets supplemented with tryptophan or glutamic acid alone had the lowest but similar growth rates ($P<0.01$). A comparison of the amino acid analysis of lathyrus (Table 11) in the present study with the amino acid requirements of chicks (NRC, 1977) indicates that the limiting amino acids in lathyrus should be the sulphur-containing amino acids (methionine and cystine). The results from the present experiment support this conclusion. Malik *et al*. (1967) have also reported a significant improvement in FCE and dressed body weight when broiler chicks were fed a 25% lathyrus containing diet supplemented with methionine as compared to that obtained with the unsupplemented diet. However, no significant ($P>0.05$) improvement with increased levels (i.e. 0, 0.29, 0.58 and 0.87 g DL-methionine/kg diet) of dietary methionine supplementation was observed by Latif *et al*. (1975) in the chicks fed diets containing only

Table 21. The performance of chicks fed lathyrus diets supplemented with one or more amino acids (Experiment IV)

Treatment[a]	Response criterion[b]		
	Feed intake	Weight gain	Feed:gain
	(g)	(g)	(g/g)
Soy-wheat, control (A)	119.3^{C}	61.9^{C}	1.93^{A}
Lathyrus, 0 supplemented amino acids (B)	57.2^{A}	5.4^{A}	12.58^{AB}
Lathyrus, methionine (C)	82.0^{B}	27.3^{B}	3.02^{A}
Lathyrus, tryptophan (D)	55.1^{A}	4.5^{A}	15.04^{AB}
Lathyrus, methionine, tryptophan (E)	91.1^{B}	31.6^{B}	3.06^{A}
Lathyrus, methionine, tryptophan, glutamate (F)	81.1^{B}	28.0^{B}	2.94^{A}
Lathyrus, glutamate (G)	48.0^{A}	2.9^{A}	22.81^{B}
Standard error	3.2	2.2	2.55

[a]Letters in brackets refer to diet designations in Table 5.

[b]The initial average weight of the 7-day old chicks was 69.8 $\pm$ 0.5 g. The duration of the experiment was 7 days. Means within each column not sharing a common superscript are significantly ($P<0.01$) different.

10% lathyrus. There were nevertheless 3% increases in feed intake and FCE in the chicks fed a diet supplemented with high level (0.087%) of methionine as compared to those on the unsupplemented diet. The lack of response to methionine supplementation in the lathyrus diets reported by Latif et al. (1975) may be in part attributed to the use of low concentrations of lathyrus (i.e. 10%), and adequate levels of methionine might presumably have been provided from the other dietary ingredients.

In the present investigation, the tryptophan content in lathyrus was not determined. Since Latif et al. (1975) have reported significant ($P<0.05$) improvements in the performance of the chicks fed a lathyrus (37%) diet supplemented with tryptophan, an attempt was made to examine the effect of tryptophan supplementation. The data (Table 21) suggest that there is no improvement with tryptophan supplementation alone. Nevertheless a combination of tryptophan and methionine supplementation in a lathyrus (82%) diet show an additional but not significant ($P>0.05$) increase in feed intake by 11% to those fed a lathyrus diet with only methionine supplementation. These results would suggest that tryptophan is not the first limiting amino acid in lathyrus although there is a possibility that it may be the second most limiting amino acid.

The importance of dispensible amino acids for chicks have been reported (Maruyama et al., 1975 and 1976). L-glutamic acid supplementation was shown to be beneficial as a dispensible amino acid for the young chicks. The results from the present experiment show no improvement in chick performance with the inclusion of 4% L-glutamate in a diet which would suggest that the indispensible amino acids were not limiting in that diet. Also, the difference may be attributed to the use of high

protein (20%) diet in the present study as compared to the low protein (12%) diet used in the reports of Maruyama et al. (1975 and 1976).

Experiment V

The objectives of this study were to confirm results reported in the previous experiment and to establish if L-glutamate or L-aspartate could alleviate the growth depression caused by lathyrus. Supplementing the lathyrus (82%) diets with methionine and tryptophan significantly ($P<0.01$) improved the performance of chicks (Table 22). In this experiment the low levels of methionine were as effective as the high levels in promoting chick performance. It may therefore be assumed that low levels of methionine supplementation are adequate for maximal growth of chicks. Addition of increased levels (i.e. 2, 4 and 6%) of L-glutamic acid or 5.4% of L-aspartic acid however produced no improvement in chicks over and above that obtained with methionine and tryptophan supplementation. Mehta et al. (1972) showed that the neurotoxin isolated from lathyrus behaved as a highly specific competitive antagonist of L-glutamic acid and L-aspartic acid, and these amino acids protected the yeast cells from growth depression when added to the culture media. A possible relationship of the glutamate-antagonizing properties of the lathyrus neurotoxin to its growth depressing effect in the chicks may be attributed to the fact that glutamic acid is a precursor of the inhibitory transmitter candidate GABA (Johnson, 1972). Increased levels of glutamic acid might increase the production of GABA which in turn suppress the neuroexcitatory effect of the lathyrus neurotoxin at the cellular level. However, the results from the present experiment suggest

Table 22. The performance of chicks fed lathyrus diets supplemented with different levels of amino acids (Experiment V)

Treatment[a]	Response criterion[b]		
	Feed intake	Weight gain	Feed:gain
	(g)	(g)	(g/g)
0 supplemented amino acids (A)	49.1^{A}	4.7^{A}	15.31^{B}
Low methionine, tryptophan (B)	74.0^{B}	23.1^{B}	3.21^{A}
High methionine, tryptophan (C)	75.7^{B}	24.4^{B}	3.12^{A}
Low methionine, tryptophan and 2% of glutamate (D)	74.9^{B}	23.1^{B}	3.27^{A}
Low methionine, tryptophan and 4% of glutamate (E)	72.1^{B}	23.2^{B}	3.14^{A}
Low methionine, tryptophan and 6% of glutamate (F)	67.7^{B}	20.8^{B}	3.30^{A}
Low methionine, tryptophan and 5.4% aspartate(G)	74.4^{B}	21.7^{B}	3.44^{A}
Standard error	1.9	1.2	1.38

[a] Letters in brackets refer to amino acid mix designations in Table 6.

[b] The initial average weight of the 7-day old chicks was 67.5 $\pm$ 0.4 g. The duration of the experiment was 7 days. Means within each column not sharing a common superscript are significantly ($P<0.01$) different.

that both glutamic acid and aspartic acid did not improve chick performance. It may therefore be further concluded that neurotoxins do not affect the performance of 7-day old chicks or that dietary glutamate and/or aspartate did not counteract the effects of these neurotoxins.

Experiment VI

The objective of this experiment was to further investigate the influence of autoclave treatment on the nutritional quality of lathyrus. Feed consumption of chicks fed diets containing autoclaved (5, 15, 30 and 60 minute intervals) lathyrus was significant ($P<0.01$) higher than those fed a raw lathyrus diet (Table 23). The feed intake in birds fed the autoclaved diets was also similar to that of the soy-wheat control group ($P>0.01$). These results would indicate that optimal feed intake is obtained when lathyrus was autoclaved for a period of 5 or more minutes. The pattern with regard to weight gain and FCE was somewhat different. There were not only significant ($P<0.01$) improvements in weight gain and FCE when ground lathyrus were autoclaved for 5 minutes but additional improvements ($P>0.01$) also occurred with longer autoclaving periods. There was an 18% increase in FCE of chicks fed a diet containing lathyrus which had been autoclaved for 30 minutes as compared to those fed a raw lathyrus diet. A similar observation was also obtained in Experiment III. The overall trend for feed:gain ratio which is inversely related to that for weight gain is illustrated in Figure 1. Data in Figure 1 also depicts the change in the percentage of insoluble nitrogen. These results show that there is a close inverse relationship between the degree of protein denaturation in lathyrus and the feed:gain ratio, or

Table 23. The performance of chicks fed diets containing lathyrus subjected to increasing autoclaving time periods (Experiment VI)

Treatment	Response criterion[a]		
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)
Lathyrus, 0 minutes autoclaving (A)	108.3^{A}	46.2^{A}	2.37^{d}
Lathyrus, 5 minutes autoclaving (B)	140.1^{B}	63.3^{B}	2.22^{c}
Lathyrus, 15 minutes autoclaving (C)	144.5^{B}	71.7^{C}	2.02^{b}
Lathyrus, 30 minutes autoclaving (D)	143.1^{B}	75.3^{C}	1.90^{b}
Lathyrus, 60 minutes autoclaving (E)	145.6^{B}	76.5^{C}	1.91^{b}
Soy-wheat, control (F)	141.7^{B}	87.2^{D}	1.63^{a}
Standard error	2.9	2.0	0.04

[a]The initial average weight of the 7-day old chicks was 56.7±0.4 g. The duration of the experiment was 9 days. Means within each column not sharing a common non-capitalized or capitalized superscript are significantly different at $P<0.05$ or $P<0.01$, respectively.

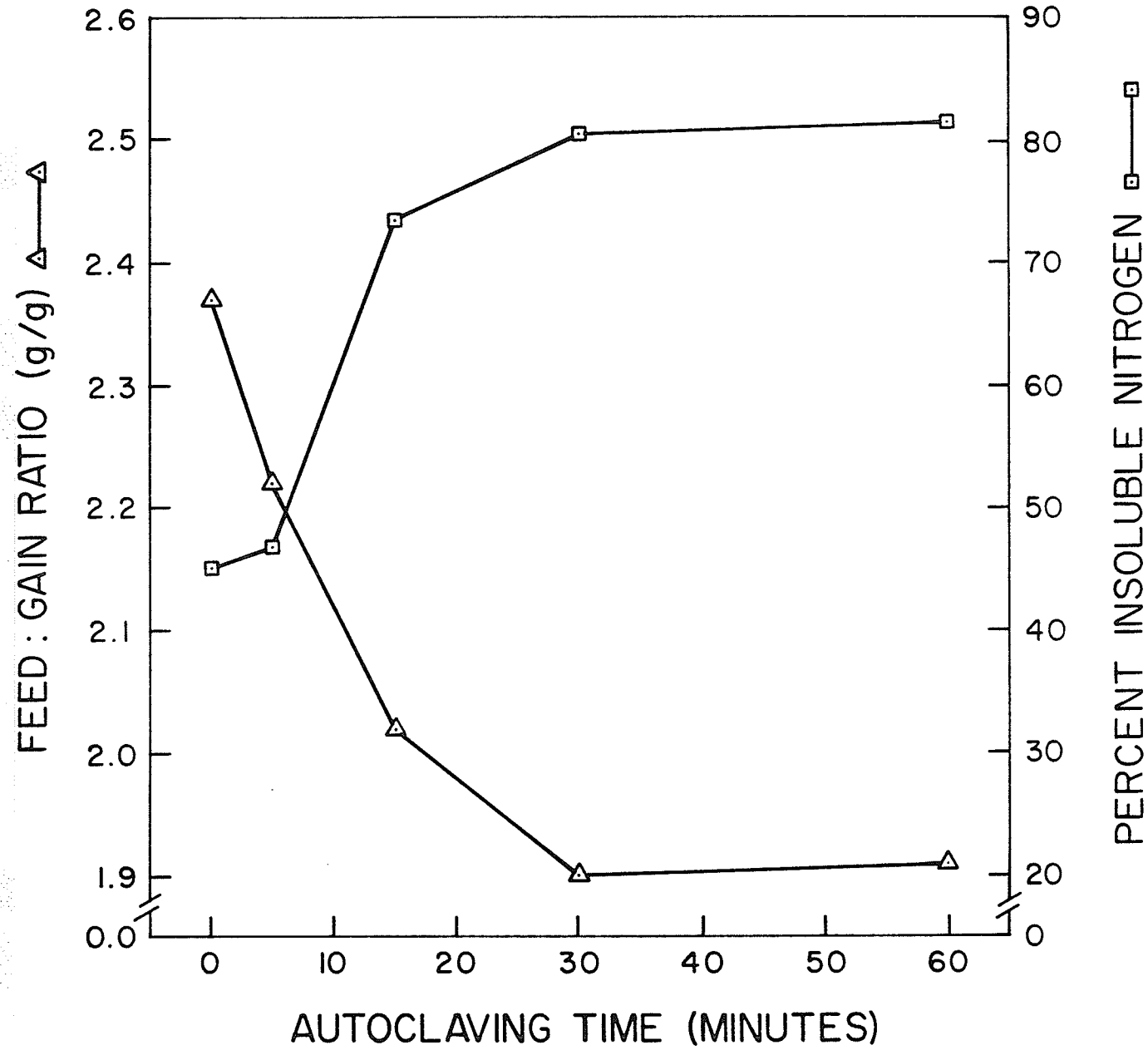

Fig. 1. Feed : Gain Ratio of Leghorn Cockerels Fed Lathyrus and the Percent Insoluble Nitrogen in Lathyrus Subjected to Increasing Autoclaving Time Periods.

that there would be a close direct relationship between FCE and level of denatured protein. These findings would suggest that the nutritive value of denatured protein and other heat treated nutrients in lathyrus are greater than that of the native components. This may be attributed to a greater digestibility of denatured protein and/or heat treated starch. Similar results have been reported by Marquardt and Ward (1979) with fababeans. Another possibility is that lathyrus may contain a heat sensitive antinutritional factor such as hemagglutinins (Latif et al., 1975) and/or trypsin inhibitors (Roy and Rao, 1971) that interfere with nutrient utilization in a manner similar to those present in soyabeans (Liener, 1955; Rackis, 1965; Lis et al., 1966 and Liener, 1974). Overall it may be concluded that lathyrus contains a heat labile factor that reduces nutrient utilization. It would also appear that lathyrus contains a separate appetite depressing factor that is highly heat sensitive. This latter factor is more readily inactivated than the former one.

Experiment VII

The objective of this study was to determine if the antinutritional factor(s) could be extracted with a 50:50 acetone-water solution from lathyrus. The nutritional value of lathyrus should therefore be improved when the growth inhibitory factor(s) is removed and the nutritional value of the corresponding soy-wheat diet containing the lyophilized acetone extract should be depressed accordingly. The results from this experiment (Table 24) show that feed intake was not increased when the acetone soluble components were extracted from lathyrus. Also feed intake was not depressed in the chicks fed a soy-wheat diet plus the lyophilized acetone extract. These results would indicate that acetone extraction

Table 24. The performance of chicks fed diets containing a lyophilized acetone extract of lathyrus and acetone-extracted lathyrus (Experiment VII)

Treatment[a]	Response criterion[b]		
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)
Lathyrus (A)	95.1^{b}	47.3^{A}	2.02^{c}
Acetone-extracted lathyrus (B)	88.5^{a}	50.2^{A}	1.77^{b}
Soy-wheat, control (C)	109.0^{c}	68.7^{B}	1.59^{a}
Soy-wheat, 8% acetone extract (D)	105.8^{c}	63.7^{B}	1.66^{ab}
Soy-wheat, amino acids ≡ 8% acetone extract (E)	103.6^{c}	66.5^{B}	1.56^{a}
Standard error	1.9	2.0	0.04

[a]Letters in brackets refer to diet designations in Table 7.

[b]The initial average weight of the 8-day old chicks was 60.0±0.5 g. The duration of the experiment was 7 days. Means within each column not sharing a common non-capitalized or capitalized superscript are significantly different at $P<0.05$ or $P<0.01$, respectively.

did not inactivate or extract the appetite depressing factor in lathyrus. Also the weight gain was not affected ($P>0.01$) by the extraction procedure, whereas there was a significant ($P<0.05$) improvement (12%) in FCE for the acetone-extracted lathyrus fed birds as compared to those fed the ground lathyrus diet. The addition of the acetone extract to the soy-wheat diet, however, did not markedly affect the feed conversion. This was true whether comparisons were made with the soy-wheat control diet (Diet C) or the soy-wheat diet that contained added amino acids equivalent to those present in the extract (Diet E). These results would indicate that acetone extraction improved the nutritional value of lathyrus by partial removal or inactivation of the antinutritional factor(s) but its antinutritional activity was subsequently lost. This inactivation may have occurred when the extract was concentrated in the cyclone evaporator or when the aqueous portion was lyophilized. However, results in Table 15 and 16 of subsection B showed that acetone-water fractionation might not be efficient enough to extract the antinutritional factor(s) from lathyrus as compared to the water extraction (no pH adjustment) procedure. It therefore may be concluded that the acetone fractionation procedure must be modified or that an alternate procedure must be employed if an active factor is to be obtained in the isolate.

The results of this study would further suggest that the growth depressing factor(s) is not a neurotoxin such as ODAP as similar isolation procedures have been employed by other researchers (Rao et al., 1964, Murti et al., 1964, Nagarajan et al., 1965) to obtain the active compound in the isolation of the neurotoxins from lathyrus.

Experiment VIII

In the previous study (Experiment VII) the antinutritional factor(s) isolated from lathyrus with 50:50 acetone-water solution was suggested to be inactivated during the concentrating process. The objectives of this experiment were to study the effects on chick performance of lathyrus after water extraction (no pH adjustment) and to determine if the extract could still be active after the isolation and freeze drying processes. The results (Table 25) show that feed intake and weight gain of chicks fed the lathyrus based diets was significantly ($P<0.01$) lower than those fed the soy-wheat based diets. The increases in body weight gain ($P<0.01$), FCE ($P<0.05$) and excreta pH ($P<0.01$) in chicks fed a diet containing water extracted lathyrus as compared to those fed the untreated lathyrus were 26, 19 and 3% respectively. In contrast, comparable feed intake values were obtained for birds fed the three soy-wheat based diets. However, body weight gain and FCE in chicks fed a soy-wheat plus the lyophilized water extract diet were 14% ($P<0.01$) and 16% ($P<0.05$) lower than those fed the soy-wheat plus supplemental amino acids equivalent to the extract diet. Moreover there was a 4% increase in excreta pH values of chicks fed the lathyrus water extract.

The retention of dry matter and crude protein (Nx6.25) are presented in Table 26. The lathyrus fed birds had a lower retention of both dry matter ($P<0.01$) and crude protein ($P<0.01$) than birds fed either the control soy-wheat diet or the soy-wheat diet that contained added amino acids. There was however no significant difference ($P>0.01$) in the retention of nutrients by the chicks fed the two lathyrus diets whereas both the retention of dry matter and crude protein by the chicks fed the

Table 25. The performance of chicks fed diets containing a lyophilized water extract of lathyrus and water-extracted lathyrus (Experiment VIII)

Treatment[a]	Response criterion[b]			
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)	Excreta pH
Lathyrus (A)	94.8^{A}	38.4^{A}	2.48^{d}	6.06^{A}
Water-extracted lathyrus (B)	96.5^{A}	48.4^{B}	2.00^{c}	6.25^{B}
Soy-wheat, control (C)	112.5^{B}	61.4^{C}	1.83^{ab}	6.30^{B}
Soy-wheat, 18% water extract (D)	111.1^{B}	57.7^{C}	1.93^{bc}	6.52^{C}
Soy-wheat, amino acids ≡ 18% water extract (E)	111.8^{B}	67.0^{D}	1.67^{a}	6.27^{B}
Standard error	1.7	1.3	0.04	0.03

[a] Letters in brackets refer to diet designations in Table 8.

[b] The initial average weight of the 8-day old chicks was 71.2±0.4 g. The duration of the experiment was 7 days. Means within each column not sharing a common non-capitalized or capitalized superscript are significantly different at $P<0.05$ or $P<0.01$, respectively.

Table 26. The retention of dry matter and crude protein by chicks fed diets containing a lyophilized water extract of lathyrus and water-extracted lathyrus (Experiment VIII)

Treatment[a]	Nutrient retained[b] (%)	
	Dry matter	Crude protein
Lathyrus (A)	66.0^{A}	45.4^{A}
Water-extracted lathyrus (B)	63.2^{A}	48.3^{A}
Soy-wheat, control (C)	73.4^{B}	56.5^{B}
Soy-wheat, 18% water extract (D)	64.5^{A}	47.4^{A}
Soy-wheat, amino acids = 18% water extract (E)	75.0^{B}	60.9^{B}
Standard error	0.7	1.1

[a]Same as those in Table 25.

[b]Not corrected for endogenous losses. Means within each column not sharing a common superscript are significantly ($P<0.01$) different.

lyophilized lathyrus water extract was markedly lower than those fed a diet that contained soy-wheat plus added amino acids equivalent to the extract. Also the deleterious effect of the water extract in chicks was reflected by the decrease in the retention of nutrients to a level similar to that obtained in the lathyrus based diets. These observations would suggest that the antinutritional factor(s) appear to depress nutrient retention and that it is partly water extractable. It may also be hypothesized that either the water-extracted lathyrus or water extract of lathyrus could alter the gut microflora status in the chicks as reflected by the increase in excreta pH values. Moreover, the appetite depressing factor in lathyrus, if present, is not extracted by water. Further retention studies are needed to substantiate the observations from the current experiment.

Experiment IX

The objectives of this experiment were to examine the effects of autoclave and/or water extraction of lathyrus, two different kinds of water extraction procedures and to determine the feasibility of the use of a retention index to monitor the antinutritional factor(s) in lathyrus. The results (Table 27) show no significant ($P>0.05$) difference in the performance of chicks fed diets containing untreated, autoclaved and water extracted lathyrus. There was also no significant ($P>0.05$) depression in chicks fed the lyophilized water extract. The data although not significantly different, follows the same trend as obtained previously. The difference between results of the autoclaved trials in the current experiment and the previous experiment may be attributed to

Table 27. The performance of Leghorn cockerels fed diets containing two lyophilized water extracts of lathyrus, raw and autoclaved samples of lathyrus, water-extracted lathyrus, soyabean meal and wheat (Experiment IX)

Treatment[a]	Response criterion[b]		
	Feed intake (g)	Weight gain (g)	Feed:gain (g/g)
Lathyrus (A)	94.5^{cd}	42.3^{c}	2.25^{b}
Autoclaved lathyrus (B)	102.1^{de}	46.5^{c}	2.19^{b}
Water-extracted lathyrus (C)	89.4^{c}	41.5^{c}	2.16^{b}
Autoclaved water-extracted lathyrus in C (D)	69.7^{a}	19.7^{a}	3.64^{c}
Soy-wheat, control (E)	109.1^{e}	58.4^{ef}	1.88^{ab}
Autoclaved soy-wheat (F)	102.2^{de}	48.5^{cd}	2.11^{ab}
Soy-wheat, 18% water extract (G)	111.3^{e}	58.0^{ef}	1.92^{ab}
Soy-wheat, amino acids ≡ 18% water extract in G (H)	108.5^{e}	63.4^{f}	1.71^{a}
Soy-wheat, 18% water water extract (pH = 5) (I)	110.5^{e}	54.4^{de}	2.03^{ab}
Water extract lathyrus (pH = 5) (J)	79.6^{b}	34.3^{b}	2.33^{b}
Soy-wheat, titrable acid in J (K)	105.9^{e}	56.0^{ef}	1.90^{ab}
Standard error	2.9	2.1	0.10

[a] Letters in brackets refer to diet designations in Table 9.

[b] The initial average weight of the 8-day old chicks was 65.8±0.5 g. The duration of the experiment was 7 days. Means within each column not sharing a common superscript are significantly ($P<0.05$) different.

the use of lower level of lathyrus (60 versus 82%) and higher percentage of fish meal (10.0 versus 7.6%) in the lathyrus diets in the present experiment as compared to that in the former experiment. The variation in the magnitude of effects in the current experiment as compared to the previous experiment for the water extraction (with no pH adjustment) trials may also be caused by the higher FCE in chicks fed the untreated lathyrus diet and lesser improvement in chicks fed the water extracted lathyrus diet in the present study.

One of the objectives of this experiment was to study the synergistic effect of autoclaving and water extraction in the utilization of lathyrus by the chicks. Results from the present experiment show significant ($P<0.05$) decreases in the performance of chicks fed a diet that contained the autoclaved water extracted lathyrus as compared to those fed the untreated lathyrus diet, nevertheless there were significant ($P<0.01$) increases in the digestibilities of amino acids by the chicks fed the autoclaved water extracted lathyrus diet compared to those fed the raw lathyrus diet (Table 28). These improvements were also similar to those fed the water extracted lathyrus. There was, however, a decrease in nitrogen retention by the chicks fed the autoclaved water extracted lathyrus diet compared to other lathyrus diets. These results would suggest that the negative growth responses obtained from the combination of autoclave and water extraction of lathyrus may be attributed to an amino acid deficiency. Since heat has long been recognized as a factor lowering the nutritive value of many foodstuffs, particularly those rich in proteins and containing reducing groups, (McNab, 1975) the consecutive treatments of water extraction and autoclave might have not

Table 28. The retention of dry matter, fat, nitrogen, total amino acids and individual amino acids by chicks fed different lathyrus and soy-wheat based diets (Experiment IX)

Treatment[a]	Nutrient retained[b] (%)																	
	Dry matter	Fat	Nitrogen	AA (total)	Ala	Arg	Asp	Glu	His	Ileu	Leu	Lys	Phe	Pro	Ser	Thr	Tyr	Val
Lathyrus (A)	64.2^{B}	89.9^{A}	74.7^{B}	80.0^{A}	81.8^{A}	72.4^{A}	80.9^{A}	86.0^{A}	74.4^{A}	81.1^{A}	82.9^{A}	67.0^{A}	82.7^{A}	81.2^{A}	77.7^{A}	76.4^{A}	81.1^{AB}	84.6^{B}
Autoclaved lathyrus(B)	71.3^{C}	94.0^{B}	71.4^{B}	87.2^{C}	89.5^{C}	79.9^{B}	84.6^{B}	91.4^{C}	78.1^{A}	92.5^{C}	93.0^{C}	76.1^{C}	91.8^{C}	88.6^{B}	87.2^{B}	85.1^{B}	90.1^{D}	90.8^{C}
Water-extracted lathyrus (C)	66.4^{B}	86.0^{A}	79.8^{C}	85.9^{BC}	87.7^{BC}	80.5^{B}	86.5^{B}	89.3^{BC}	78.2^{A}	87.7^{B}	88.6^{B}	77.5^{C}	87.5^{C}	86.8^{B}	84.9^{B}	82.1^{AB}	85.5^{BCD}	90.2^{C}
Autoclaved water-extracted lathyrus in C (D)	64.0^{B}	90.3^{A}	61.0^{A}	84.3^{B}	85.2^{B}	80.5^{B}	86.3^{B}	88.4^{B}	68.5^{A}	88.0^{B}	88.2^{B}	76.0^{C}	86.4^{B}	82.8^{A}	83.6^{B}	80.9^{AB}	82.0^{ABC}	86.4^{B}
Soy-wheat, control (E)	69.9^{C}	87.6^{A}	83.9^{CD}	88.1^{C}	86.5^{B}	90.4^{C}	84.9^{B}	91.2^{C}	84.3^{B}	87.2^{B}	88.1^{B}	88.2^{D}	88.0^{B}	89.5^{B}	85.4^{B}	82.6^{AB}	85.4^{BCD}	89.3^{C}
Soy-wheat, 18% water extract (G)	61.4^{A}	88.2^{A}	72.7^{B}	80.1^{A}	80.2^{A}	73.8^{A}	78.3^{A}	86.3^{A}	70.1^{A}	81.9^{A}	82.7^{A}	71.2^{B}	82.7^{A}	83.2^{A}	78.3^{A}	75.9^{A}	78.1^{A}	81.3^{A}
Soy-wheat, amino acids = 18% water extract in G (H)	69.2^{C}	88.4^{A}	83.5^{D}	88.4^{C}	86.0^{B}	91.5^{C}	86.5^{B}	91.3^{C}	84.9^{B}	88.1^{B}	88.2^{B}	88.9^{D}	88.2^{B}	88.7^{B}	85.1^{B}	82.9^{AB}	87.2^{CD}	89.4^{C}
Standard error	0.62	0.87	0.89	0.54	0.63	0.98	0.69	0.49	2.34	0.58	0.54	0.86	0.57	0.54	0.97	1.30	1.14	0.55

[a] Letters in brackets refer to diet designations in Table 9.

[b] Not corrected for endogenous losses. Means within each column not sharing a common superscript are significantly ($P<0.01$) different.

only extracted certain essential amino acids but also might have destroyed amino acids in proteins with free amino groups such as lysine, tryptophan, histidine and arginine by the Maillard or browning reaction. This is also supported by the divergency in amino acid digestibilities and nitrogen retention values by the chicks fed the autoclaved water extracted lathyrus as compared to those fed the autoclaved lathyrus diet in the present study.

Results from the amino acid analyses of the different lathyrus extracts (Table 15) and the percentage of non-protein nitrogen in the different extracts (Table 16) have shown that the lathyrus water extract obtained under acidic condition had a similar concentration of non-amino acid nitrogen to that obtained under neutral condition. The results from Table 27 of the present experiment indicate that water extraction of lathyrus under either acidic or neutral conditions affected the chick performance in a similar manner. Unfortunately, retention analyses were not carried out for chicks fed lathyrus after extraction under acidic condition.

Results from the retention study show that autoclave and/or water extraction of lathyrus increased the digestibilities of amino acids by the chicks as compared to those fed on the untreated lathyrus diet. Water extraction had no effects on the retention of dry matter by the chicks but a significant ($P<0.01$) improvement in nitrogen retention (7%) occurred. This observation was similar to that in the previous study (Experiment VIII). On the other hand autoclave treatment of lathyrus increased (11%) the retention of dry matter to a level that was similar to the dry matter retention value for the soy-wheat control

group. This may be attributed to the increase in digestible carbohydrates, protein and fat in lathyrus after autoclave treatment. Marquardt and Ward (1979) also have reported an increase of 23% for dry matter retention and 8% for total amino acid digestibilities in the birds fed autoclaved versus raw fababeans that were free of condensed tannins.

In the present study, the retention values of birds fed the untreated lathyrus and soy-wheat control diets, respectively, were 64 and 70% for dry matter, 75 and 84% for nitrogen and 80 and 88% for total amino acids. The patterns of the retention of individual amino acids in birds were similar to that of the total amino acids. The retention values for individual amino acids ranged from a low of 67% (lysine) for the raw lathyrus diet and 83% (threonine) for the soy-wheat control diet to a high of 86% and 91% respectively with regard to glutamic acid digestibilities. The observation that water extraction improved total amino acid digestibilities of lathyrus almost to that of the soy-wheat control group whereas the addition of the lyophilized lathyrus water extract to the soy-wheat diet reduced total amino acid retention to that of raw lathyrus indicate that the antinutritional factor(s), which depresses amino acid retention and is possibly the same factor(s) that reduced growth in the previous studies, can be quantitatively extracted from lathyrus. Also its antinutritional properties can be preserved using techniques similar to those used in the current experiment. It is possible that even greater activity may be obtained if the antinutritional factor was extracted in warm rather than boiling distilled water (refer to subsection E II in Materials and Methods section). These observations should form a basis for future fractionation studies in an attempt to

more clearly identify the growth depressing compounds. Moreover in the current study, the amino acids whose retention show the greatest improvement resulting from either autoclave or water extraction was lysine (14%) followed by isoleucine, leucine, serine and threonine; that showing the least response was histidine (5%), followed by valine, phenylalanine and alanine. The antinutritional factor(s) in lathyrus could, therefore, be monitored by following the retention of only one or two amino acids such as lysine and/or isoleucine.

Future studies should be carried out to more clearly define the thermal stability characteristics of the antinutritional factor(s) in lathyrus and to determine whether the factor(s) occurs in nature as a protein or non-protein. These studies would be facilitated by the development of an extraction procedure that would not only maximize the extractability of the antinutritional factors but also preserve native proteins.

GENERAL DISCUSSION

The potential of lathyrus as a protein crop for poultry is considerable. Lathyrus has yields comparable to that of field peas (Furgal, J., personal communication) and its drought resistance ability makes lathyrus well adapted to the limited moisture growing areas in Canada. The rapid germination rate of lathyrus, as compared to that of field peas, enables good production in areas of southern Manitoba where the precipitation during the growing season (from May 1 to September 30) is low (30 to 36 cm) (Shaykewich, 1974). The proximate analysis and amino acid composition of lathyrus (Table 10 and 11) would suggest that as an animal feed ingredient lathyrus on an equal protein basis should support growth to the same extent as soyabean meal, fababeans or field peas.

Although its proximate analysis would suggest that lathyrus should be a good feedstuff for poultry, feeding trials indicate that it is deficient in certain nutrients and appears to contain antinutritional factors. The results from the current study demonstrated that the first limiting amino acid in lathyrus was methionine with a slight possibility that tryptophan was the second limiting amino acid. These results agree with those of Malik *et al.* (1967) but are contrary to those of Latif *et al.* (1975) who reported no beneficial response to methionine supplementation. This latter author reported that tryptophan was the limiting amino acid. The observation that methionine was the first limiting amino acid is also consistent with amino acid analysis data and data by other researchers who reported that methionine is also the first limiting amino acid in other legumes such as soyabeans

(Scott et al., 1976) and fababeans (Marquardt and Campbell, 1974).

The results from the current study also demonstrated that chick performance progressively decreased as the level of lathyrus in the diet was increased. Feeding of lathyrus over a longer period of time did not cause an increased incidence of death relative to the control diet as reported by Panda et al. (1972), in fact the lathyrus fed birds appeared to improve their relative performance as the duration of the experiment increased in the present investigation. These observations suggested that lathyrus was not toxic to the birds and that the birds appeared to adapt to some of its adverse effects. Increasing both energy and protein density of the diet improved growth performance in lathyrus fed birds to a greater degree than in soy-wheat fed birds which suggested that these two major classes of nutrients were less available in lathyrus than in a soy-wheat diet.

Autoclave treatment of lathyrus greatly improved its nutritional value. Heat treatment for short periods (5 minutes) of time improved feed intake to a much greater degree than FCE while heat treatment for longer period of time (30 minutes) resulted in the reverse pattern. The latter response which resulted in increased FCE and digestibilities of both dry matter and protein may be attributed to two different effects. Autoclave treatment may partially gelatinize lathyrus starch and denature lathyrus protein which would improve their digestibilities and/or it may inactivate certain proteinaceous inhibitors such as trypsin inhibitor or phytohemagglutinin that may interfere with nutrient digestibility. These conclusions are supported by the observation that there appears to be a close association between improved FCE and its amount of denatured

protein in the current study. These observations are also consistent with those of Marquardt and Ward (1979) who reported that the improved utilization of autoclaved fababeans may be attributable to both inactivation of an antinutritional factor (condensed tannins) and a thermal effect on both protein and starch. These results suggested that there may be two types of antinutritional factors, one of which is an appetite depressing factor and the second one is a factor that interferes or is associated with reduced nutrient utilization.

Attempts to extract the active compounds using different solvent systems support the hypothesis that lathyrus contains a thermolabile factor that depresses nutrient utilization. For example, lyophilized neutral water extract of lathyrus when added to a soy-wheat diet caused a depression in FCE and digestibilities of nutrients particular amino acids. The amino acid that seemed to be most dramatically affected was lysine, those being least affected were glutamic acid, leucine, phenylalanine and alanine. There were also corresponding improvements in nutrient utilization by the chicks fed extracted lathyrus as compared to those on raw lathyrus. These results would suggest that the active compound can be partially removed from lathyrus and that its activity can be preserved in samples that are mildly processed.

The results also demonstrated that it was not possible to obtain an active extract when the 50:50 acetone:water or acidic aqueous extraction techniques were employed. In these procedures the amount amino acid nitrogen (i.e. protein) that was extracted per unit weight of lathyrus was greatly depressed. This depression was attributed to the low extractability of protein at pH 5 and to the denaturation of protein in

the acetone solution. However, there was a relative increase in the concentration of non-amino acid nitrogen containing compound. It may be assumed that some of these would be ODAP, L-ODAP or DAPN, compounds that have been implicated as causative agents of certain neurological diseases. Other authors using similar extraction techniques have obtained active forms of these compounds (Rao et al., 1964; Bell and O'Donovan, 1966 and Rukmini, 1968 and 1969). Since the acetone extract appeared to contain these compounds but did not affect animal performance, it may be concluded that these compounds are not responsible for the depressed performance of chicks fed lathyrus containing diet. The experiments with autoclaved lathyrus treatment also appear to support this conclusion as these compounds may be resistant to heat treatment. Although the neurolathyrogens (i.e. ODAP, L-ODAP and DAPN) did not appear to affect chick performance they did seem to increase chick hypersensitivity to some degree.

Information obtained in these studies should facilitate future attempts to identify the active growth inhibitory compounds. For example, most of the studies were designed to test the hypothesis that the neurotoxins were the agents responsible for poor chick performance. As a result diets were formulated to be high in both protein and energy so as to assure that they would not be limiting. As indicated above this hypothesis seems to be wrong and in fact the main limiting factors, due to the presence of the antinutritional factor, in lathyrus is energy and protein. The sensitivity of the animal performance data including amino acid retention would therefore be considerably improved if both the energy and protein levels of the diets were maintained at the

minimum level of the nutrient requirements of chick (NRC, 1977). The effects of the antinutritional factor could also be readily monitored by following amino acid digestibilities. Since all amino acids appear to be affected to varying degrees, it may only be necessary to examine the effect on a single but sensitive amino acid such as lysine.

The extraction techniques utilized in this study can also be improved by reducing the temperature of the extracting solvent. The use of boiling water in all extraction procedures, which were initially employed to enhance the extraction of neurotoxins, may have a detrimental effect on proteins and therefore probably reduce the actual yield and activity of the antinutritional factors.

It may be concluded that methionine is the first limiting amino acid in lathyrus and that lathyrus contains one or possibly two thermolabile factors. The first factor appears to be non-extractable and to have an appetite depressing effect. The second factor depresses nutrient utilization, particularly protein and can be extracted in aqueous solution at a neutral pH. The factors also do not seem to be neurolathyrogens.

Additional studies should be carried out to identify and characterize the two nutritional factors, to more clearly establish the nature of the apparent adaptation by chicks to lathyrus, to establish optimal processing methods and to establish the metabolizable energy and amino acid availabilities of lathyrus when subjected to different processing methods.

SUMMARY AND CONCLUSIONS

A series of experiments was conducted with growing Leghorn cockerels in the evaluation of lathyrus (Lathyrus sativus Linn. var. seminis albi) as a feedstuff for poultry, the findings of these experiments can be summarized as follows:

1. Lathyrus, relative to the requirements of chicks, is rich in lysine and contains relatively adequate concentrations of other essential amino acids, but is deficient in sulphur-containing amino acids and possibly tryptophan.
2. Proportional decreases in feed consumption, body weight gain and FCE were observed in chicks fed increasing levels (i.e. 25, 50 and 75%) of lathyrus in the diet.
3. Chicks seem to adapt to the growth depressing effects of lathyrus.
4. Both energy and protein are less available in lathyrus than in wheat and soyabean meal.
5. The poor performance of the lathyrus fed birds cannot be solely attributed to an appetite effect.
6. Supplementation of different levels of dicarboxylic amino acids did not alleviate the growth depressing effect of lathyrus.
7. Autoclaving for short period (5 minutes) improved the palatability of lathyrus to a much greater degree than FCE while heat treatment for longer period of time (30 minutes) resulted in the reverse pattern.

8. Water extraction under neutral pH condition seemed to remove some of the antinutritional factors from lathyrus, and the activity of this extract caused growth depressions when added to a soy-wheat based diet.
9. Acetone:water fractionation was not successful in the removal of the thermolabile growth inhibitors from lathyrus.
10. Neurolathyrogens did not appear to be the antinutritional factors.

These preliminary results would suggest that lathyrus if properly processed can be used as a poultry feed. However, a considerable amount of additional research should be conducted to identify the nature of the growth depressing factors and to devise dietary feeding regimes that neutralize or minimize the adverse effects of lathyrus.

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