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