# THE EVALUATION AND ENHANCEMENT OF THE NUTRITIVE QUALITY OF PEAS (*PISUM SATIVUM*) FOR POULTRY

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Francis Adegbaye Igbasan

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THE UNIVERSITY OF MANITOBA

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# THE EVALUATION AND ENHANCEMENT OF THE NUTRITIVE QUALITY OF PEAS (PISUM SATIVUM) FOR POULTRY

BY

FRANCIS ADEGBAYE IGBASAN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

## DEDICATION

To my mother Mrs. Dorcas Olatunwo Igbasan and to all mothers who toil everyday for the betterment of their children.

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It must not be presumed that the task of becoming what one wants to be in life is an easy one except through the grace of God. My appreciation and thanks go to my adviser Dr. W. Guenter for his fatherly encouragement, guidance and untiring assistance throughout this research and my programme of study. I would like to thank Dr. R.R. Marquardt, Dr. J.R. Ingalls, Dr. G.H. Crow, and Dr. G.M. Ballance for serving on my advisory committee. The external examiner, Dr. R. Blair is acknowledged for taking time to review this thesis.

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## ABSTRACT

The purpose of this study was to evaluate and enhance the nutritive quality of peas for poultry. Twelve cultivars of peas; 8 yellow peas (YP), 2 green peas (GP), and 2 brown peas (BP) were evaluated for differences in chemical composition and digestibility in chickens. The cultivars displayed wide variations in CP, AA and starch contents which were not related to the seed coat colours. The BP cultivars contained appreciable quantities of tannins while the YP and GP cultivars were devoid of tannins. The cultivars were relatively high in metabolizable energy and the digestibility value for individual AAs was comparable to that of soybean meal and canola meal. There was a trend towards lower AA digestibility in the BP cultivars. The study showed that location and nitrogen fertilization influenced CP content and AA composition of peas. Three cultivars (Impala, Radley and Sirius) representing YP, GP and BP were selected for use in the broiler and laying hens diets. The cultivars were included in broiler chick diets at 0, 100, 200 and 400 g/kg and in laying hen diets at 0, 200, 400 and 600 g/kg. All diets were formulated to meet NRC requirements for broilers and layers respectively. The inclusions of up to 200 g/kg for broiler diets and 400 g/kg for layer diets did not affect performance. When peas comprised 400 g/kg of broiler diets and 600 g/kg of layer diets performance was adversely affected. Micronization of peas at a temperature between 110 and 115 C for 55 s significantly improved nutrient digestibilities, however, the digestibility of lysine was decreased. The performance of broilers and layers fed micronized peas at 400 and 600 g/kg, respectively, was similar to those fed the wheat/soybean meal control diets. Dehulling was only beneficial when applied to BP which contained tannins. Supplementing pea-based diets with pectinase enzyme improved weight gain and feed consumption but not feed conversion of broiler chicks, however, laying hens did not respond to pectinase supplementation.

## FOREWORD

This thesis was prepared following a manuscript format. There are seven manuscripts divided into three chapters, chapters three, four and five. Chapter three contains manuscripts 1 & 2, chapter four manuscripts 3, 4 & 5 while chapter five contains manuscripts 6 & 7. With the exception of manuscript 1, all other manuscripts have been published or accepted for publication in different journals indicated below each manuscript. For the purpose of uniformity, all manuscripts were formatted to meet Poultry Science Guidelines for manuscript preparations, however, no changes were made to the content of each manuscript.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| AA | Amino acid |
| AOAC | Association of Official Analytical Chemists |
| APD | Apparent protein digestibility |
| AMEn | Nitrogen-corrected apparent metabolizable energy |
| CP | Crude protein |
| DM | Dry matter |
| GPC | Green pea chips |
| IU | International unit |
| NDF | Neutral detergent fibre |
| NRC | National Research Council |
| NSP | Non-starch polysaccharides |
| SAS | Statistical analysis system |
| SBM | Soybean meal |
| SD | Standard deviation |
| SEM | Standard error of the mean |
| TME | True metabolizable energy |
| TMEn | Nitrogen-corrected true metabolizable energy |
| YPC | Yellow pea chips |

# CHAPTER ONE

## INTRODUCTION

Peas (*Pisum sativum* L.) are a legume crop which is well adapted to temperate climate. The production of peas is on the increase especially in Europe and Canada. In Canada, pea producers have witnessed a 7-8 fold increase in pea production in the last decade (Slinkard, 1994). Because of this increase in production coupled with improvements in breeding strategies, a number of new cultivars have been developed and registered in Canada. Among the newly registered cultivars are Fluo, Montana, Baroness, Highlight, Trump, Titan, Sirius, Radley, Impala, Carman and Express.

Despite this dramatic increase in production, peas remain an under-exploited feedstuff in poultry nutrition. Poultry diets in Canada are still based on wheat, barley or corn and soybean meal as the major protein supplement. Soybeans are not locally produced but are imported. One logical approach to limiting dependency on soybeans and possibly reducing cost of production is the utilization of indigenous protein sources like canola and peas. Currently canola meal is gaining some acceptance in the feed industry but its utilization is limited because of high fibre and low energy contents (Bell, 1993). Peas are likely to become an increasingly important home-grown protein source not only for poultry but also for swine in Canada.

Lack of adequate nutritional information may, in part, be responsible for low usage of peas in poultry diets. Data on the nutritional composition of peas in general can be found in standard feed tables (NRC, 1994). However, several studies (Holt and Sosulski,

1979; Matthews and Arthur, 1985) have shown that major differences exist in composition among pea cultivars. Nutritionists and feed manufacturers need data specific to pea cultivars available in a locality to enable them to formulate balanced diets containing peas. There is little or no data available on Canadian grown peas, particularly the newer cultivars.

In recent years, conflicting results have been published regarding the acceptable levels of raw peas in diets for broiler chickens and laying hens. Brenes *et al.* (1989) reported that broiler chicks can be grown with diets containing 80% peas. The same authors (Brenes *et al.*, 1993) demonstrated that satisfactory growth performance of chicks could be obtained at 48% peas in the diets. However, Moran *et al.* (1968) reported lower acceptable dietary levels of peas in their studies. These authors encountered significant depression in chick growth and feed utilization when peas constituted 35% of chick diets which implies that chicks could not tolerate high levels of peas in their diets.

Moran *et al.* (1968) found that 15 or 30% peas with methionine supplementation had no effect on egg production but resulted in a significant decrease in feed conversion. In contrast, Davidson (1977 and 1980a) reported that when laying hen diets contained 17 and 30% peas, production performance was 20 and 45% lower, respectively, than the fishmeal control diet. In a subsequent study, Davidson *et al.* (1981) also demonstrated that egg production was reduced by 15% with 40% peas in the diets supplemented with adequate methionine. More recently, Ivusic *et al.* (1994) has shown that egg production and feed conversion were not affected when peas were incorporated in diets at levels up to 59% but thinner egg shells were observed. These contradictory reports coupled with

the cost of peas limit the use of peas in practical poultry formulations.

When comparing the data for peas obtained from digestibility and feeding trials (Moran *et al.*, 1968; Davidson *et al.*, 1981; Longstaff and McNab, 1987) to their chemical composition (Savage and Deo, 1989), it is obvious that the nutritive value of peas is lower than that predicted by its composition. This indicates that raw peas may contain some antinutritional factors that could result in poor utilization by poultry. It would also suggest that there may be a potential for improving the nutritive value of peas through the use of appropriate processing technologies.

This study had a three-fold objective. First, to provide detailed knowledge on the chemical composition of peas with emphasis on the newly developed and registered cultivars in Western Canada. Second, to establish the replacement value of peas within the physical and nutritional limitations in broiler and laying hen diets. Third, to investigate methods to improve the nutritive value of peas for poultry. This research will not only provide valuable information on the utilization of peas as protein and energy supplements in poultry diets, but it will also generate more awareness among pea producers relative to alternate use of the pea crop and may further lead to an increase in crop acreage. Pea breeders will also find the research results useful for developing better varieties.

# CHAPTER TWO

# LITERATURE REVIEW

## THE ORIGIN, ADAPTATION AND PRODUCTION OF PEAS

The exact origin of the pea plant is obscure but it is believed to be one of the oldest cultivated plants. Evidence from archaeological studies shows that peas were grown in the Near Eastern and Greek neolithic farm settlements as early as 7,000 to 6,000 B.C. (Zohary and Hopf, 1973). The carbonized remains of pea seeds dating back to 7,000 B.C. have also been discovered in Switzerland (Yamaguchi, 1983). Vavilov (1926) listed different centres of origin of peas. He regarded central Asia, the Near East, Abyssinia, and the Mediterranean as the place of origin of all legumes including peas. In Canada, peas have been grown ever since farmers started plowing the prairies over 100 years ago (Slinkard, 1994). About 20,000 ha were cultivated in the years immediately after World War II with production concentrated in Manitoba (Slinkard and Blain, 1988).

The pea plant is an annual herbaceous cool season plant with either smooth (round) or wrinkled seeds. The smooth types are more adapted to cool weather conditions than the wrinkled types (Yamaguchi, 1983). Most cultivars grown in Canada are smooth-seeded types. The seed coat may be clear or pigmented while the cotyledons are either yellow or green. Peas can be grown in a wide range of soil types from light sandy loams to heavy clays but in any soil, there must be good drainage as peas do not tolerate soggy or water-soaked conditions (Yamaguchi, 1983; Ali-khan and Zimmer, 1989). In temperate

climates where winters are severe, the crop is usually planted in the spring (like in Canada) and in the late fall and early winter where there is little or no frost. In the tropics and subtropics, peas are planted at high elevations (1200-2600 m) where temperatures remain cool (Yamaguchi, 1983). Peas grow best at mean temperatures of 13-18 C.

In recent years, the production of peas in Canada has increased dramatically (Table 1). According to Slinkard (1994) the increase in production is attributed to the opening of the European feed pea market in 1985 and the resulting high prices and the increased emphasis on crop diversification, value-added processing and sustainability of agriculture. Above 80% of Canadian pea production is exported and Western Europe is the main market. The Canadian pea industry was initially based on one cultivar, century, but today over 40 new cultivars have been registered. Most of the new cultivars were developed in Europe. Pea production in Canada was initially located in Eastern Canada but because of severe disease problems, production has shifted to the prairies (Ali-Khan and Zimmer, 1989). At present Saskatchewan is the leading province in pea production and currently produces about 60% of the Canadian pea crop, followed by the province of Alberta and then Manitoba (Slinkard, 1994).

## CHEMICAL COMPOSITION OF PEAS

### *Crude Protein and Amino Acid Content*

Peas are regarded principally as a protein crop, and in assessing their suitability in monogastric nutrition the main interest is focused on the protein quantity and quality.

TABLE 1. Pea production in Canada between 1984 and 1993.

| Year | Land area cultivated (ha x 1000) | Production (metric tons x 1000) |
|---|---|---|
| 1984 | 73.6 | 131.0 |
| 1985 | 73.6 | 168.8 |
| 1986 | 129.9 | 238.9 |
| 1987 | 234.0 | 415.0 |
| 1988 | 268.0 | 319.7 |
| 1989 | 148.0 | 234.1 |
| 1990 | 122.0 | 264.0 |
| 1991 | 196.1 | 409.7 |
| 1992 | 270.0 | 504.8 |
| 1993 | 500.0 | 970.2 |

Summarized from: Slinkard (1994).

There is a wealth of data relating to the crude protein content of peas. These data are summarized in Table 2. As indicated in this table, the crude protein content of peas could vary between 15.5 to 39.7%. This broad range of protein contents could be a reflection of the conditions under which pea cultivars are grown or the inherent cultivar differences. Ali-Khan and Youngs (1973) reported a range of 22 to 32% protein content in peas and they attributed the variation to location and year. This was latter confirmed by Matthews and Arthur (1985) who also showed that cultivars differed markedly in response to environment. McLean *et al.* (1974) reported an increase in protein content of peas with the application of nitrogen fertilizer. Pea protein content increased from 20 percent to 30 percent. In addition Carre *et al.* (1991) observed higher protein contents in peas sown in spring than those peas sown in winter.

Studies have also shown that there is genetic variation in protein content of peas. Bajaj *et al.* (1971) and Reddy *et al.* (1979) reported differences in protein content among pea lines. Cousin (1983) showed that protein content between individual seeds varied from 20 to 45% for the same genotype. In addition Cousin (1983) and Matthews and Arthur (1985) found that wrinkled-seeded pea varieties were about 2 percent units richer in protein content than the round-seeded (smooth) varieties.

Arginine, aspartic acid and glutamic acid are present in large concentrations (Table 2). These three amino acids accounted for about 41% while leucine and lysine comprised another 15% and histidine, methionine, threonine, tryptophan and cystine accounted for less than 11% of the total (Holt and Sosulski, 1979). When comparing the values of essential amino acids found in pea seeds to those of standard proteins like meat

TABLE 2. Crude protein content and amino acid composition of whole seed protein and protein fractions (albumin and globulin) of peas.

| Amino acid | Whole seed | Albumin | Globulin | Vicilin[1] | Legumin[1] |
|---|---|---|---|---|---|
| Crude protein (% DM) | 15.5-39.7 | - | - | - | - |
| Amino acid (g/16g N DM) | | | | | |
| ESSENTIAL | | | | | |
| Arginine | 6.8-14.9 | 4.1 | 8.1 | 7.3 | 10.5 |
| Histidine | 1.9-4.8 | 2.9 | 2.2 | 2.1 | 2.8 |
| Isoleucine | 2.4-6.2 | 4.4 | 4.8 | 5.1 | 4.0 |
| Leucine | 4.2-10.9 | 5.2 | 8.9 | 9.2 | 8.1 |
| Lysine | 4.6-12.3 | 10.3 | 7.0 | 7.9 | 4.9 |
| Methionine | 0.8-2.8 | 1.0 | 0.6 | 0.2 | 0.7 |
| Phenylalanine | 2.9-6.9 | 4.6 | 5.5 | 6.2 | 4.9 |
| Threonine | 2.8-6.8 | 4.5 | 3.3 | 3.4 | 2.9 |
| Tryptophan | 0.7-1.9 | 1.2 | 1.0 | 0.1 | 1.1 |
| Valine | 2.8-7.0 | 5.2 | 4.9 | 4.6 | 4.6 |
| NON-ESSENTIAL | | | | | |
| Alanine | 2.8-7.4 | 5.2 | 4.0 | 3.0 | 3.7 |
| Aspartic acid | 7.8-18.9 | 11.4 | 12.0 | 12.0 | 12.5 |
| Cystine | 0.2-3.5 | 3.1 | 1.2 | 0.4 | 0.7 |
| Glutamic acid | 11.1-27.9 | 16.6 | 16.9 | 19.3 | 21.1 |
| Glycine | 2.9-7.3 | 6.4 | 3.9 | 3.1 | 3.4 |
| Proline | 2.5-6.0 | 5.4 | 4.5 | 3.5 | 4.3 |
| Serine | 2.9-7.8 | 4.1 | 4.9 | 5.8 | 4.5 |
| Tyrosine | 1.9-5.5 | 4.3 | 3.4 | 3.0 | 3.3 |

Based on data from: Bajaj *et al.* 1971; Boulter *et al.* 1973; Holt and Sosulski, 1979; Reddy *et al.* 1979; Monti, 1983; Aman and Graham, 1987; Mosse *et al.* 1987; Savage and Deo, 1989; Leterme *et al.* 1990; Lalles, 1993; Brunsgaard *et al.* 1994; Kosson *et al.* 1994.

[1] Components of globulin.

meal, cow's milk and eggs (Lalles, 1993), the limiting character of the sulphur amino acids is obvious. However, amongst the genera of *leguminosae*, only soybeans (*Glycine*) contain more sulphur amino acids than peas (Muller, 1983; Wiseman and Cole, 1988; Lalles, 1993). On the average, pea seeds are higher in sulphur amino acids than the seeds of beans (*Vicia*) and lupins (*Lupinus*). In comparison with the amino acid profiles of conventional feedstuffs, especially cereal grains (NRC, 1994), peas contain more lysine and less sulphur amino acids. In the context of meeting the dietary requirements of indispensable amino acids for poultry (NRC, 1994), cereal grains and peas are nutritionally complementary in that those amino acids deficient in one (lysine in cereals and sulphur amino acids in peas) being adequate in the other. However, despite this complementary nature, studies (Moran *et al.*, 1968; Reddy *et al.*, 1979) showed that diets based on cereal and peas could not maintain satisfactory production performance without methionine supplementation.

The concentration of amino acids in the protein of peas is a function of the storage proteins (Gueguen and Barbot, 1988). Two main types of storage proteins have been identified and characterized on the basis of their solubility. The water-soluble proteins referred to as albumins and the salt-soluble proteins as globulins (Schroeder, 1982). Storage globulins contribute up to 80 percent of the seed proteins in peas and principally consist of vicilin and legumin in the ratio of about 1.5 to 2.0 (Boulter *et al.*, 1973; Casey and Domoney, 1983). On the other hand, albumins represent 20-35 percent of the cotyledonary proteins (Schroeder, 1982). According to Johnson and Clay (1974) the proportion of the albumin fraction remains stable within one variety and is independent

of nitrogen application.

The amino acid composition (Table 2) differs between albumins and globulins and also between the vicilin and legumin fractions of globulins. The amino acid profile of albumins shows relatively high contents of sulphur amino acids and other essential amino acids compared to the globulin fractions (Schroeder, 1982; Leterme *et al,*. 1990). A much higher content of arginine occurs in the globulin fraction than in the albumin fraction. A closer look at the amino acid profiles of the albumin fraction of most genera of *leguminosae* provided by Muller (1983) reveals that some amino acids are similar to that of standard proteins and some are higher. Bajaj *et al.* (1971) found a high correlation (r = 0.99) between the biological value (determined as protein efficiency ratio) of pea protein and albumin content and they suggested that the high proportion of sulphur amino acids and lysine present in this protein fraction may be responsible for this excellent correlation. The legumin fraction of globulins contain more sulphur amino acids and tryptophan than the vicilin fraction (Boulter *et al.*, 1973).

***Carbohydrate Content***

Carbohydrates form the largest part of poultry feed. Cereal grains are the major contributors, but where starchy legumes like peas are used in poultry diets, they also make significant contribution to the carbohydrate component. Carbohydrate constituents of peas are presented in Table 3.

The free sugar content of peas is very low and most of this is sucrose (18.5-42.0 g/kg) (Cerning-Beroard and Filiatre-Verel, 1976). Like other legumes, peas contain α-

galactosides or oligosaccharides of the raffinose family. These carbohydrates which include raffinose, stachyose, verbascose and ajugose are present in the endosperm of peas at reasonable quantities (Fleming, 1981; Sosulski *et al.*, 1982; Saini, 1989). Sosulski *et al.* (1982) reported that verbascose is the predominant α-galactoside in field peas.

Because monogastric animals lack the enzyme, α-galactosidase, which is required to cleave the α-linked galactose units present in these oligosaccharides, they escape undigested to the lower intestinal tract (Saini, 1989). They are subjected to microbial fermention in the colon and caecum producing volatile fatty acids and intestinal gases such as hydrogen, carbon dioxide and methane (Fleming, 1981). The build up of these fermentation by-products may cause nausea, diarrhea, cramps and discomfort in animals. Recently, Coon *et al.* (1990) reported a 20% decrease in metabolizable energy ($TME_n$) of soybean meal in which its oligosaccharide contents were not extracted.

A third category of carbohydrate in peas are the polysaccharides. Like cereal grains, starch is the most abundant (240.0-500.0 g/kg) polysaccharide in peas. As a nutrient starch is the main dietary source of energy and these high levels therefore, indicate the importance of peas as an energy supplement in livestock and poultry feeding. The National Research Council (NRC, 1988) documented that the digestible energy content of peas for pigs is higher than that of barley and comparable to that of wheat. However, in poultry, pea starch is less digestible than starch in any of the cereal grains (Longstaff and McNab, 1987) and hence of lower metabolizable energy value (Conan and Carre, 1989). Poor digestibility of pea starch has been attributed, in part, to low accessibility of starch granules to enzymic attack, which is dependent on endosperm cell

TABLE 3. Carbohydrate content of peas

| Component | Content (g/kg DM) |
|---|---|
| FREE SUGARS | |
| Glucose | 0.4-9.0 |
| Sucrose | 18.5-42.0 |
| OLIGOSACCHARIDES | |
| Raffinose | 3.0-16.0 |
| Stachyose | 10.4-37.0 |
| Verbascose | 14.7-47.9 |
| Ajugose | 0.6-1.3 |
| POLYSACCHARIDES | |
| Starch | 240.0-500.0 |
| Non-starch(cell wall component) - simple sugar profile | |
| Rhamnose | 1.1-3.0 |
| Arabinose | 35.6-41.0 |
| Xylose | 16.0-18.0 |
| Mannose | 1.2-2.0 |
| Galactose | 9.0 |
| Glucose | 64.2-81.3 |
| Uronic acids | 36.0 |
| Lignin (non-carbohydrate) | 4.8-16.0 |

Based on data from: Cerning-Beroard and Filiatre-Verel, 1976; Reichert, 1981; Fleming, 1981; Sosulski *et al.*, 1982; Brillouet and Carre, 1983; Cousin, 1983; Wright *et al.*, 1984; Aman and Graham, 1987; Wiseman and Cole, 1988; Saini, 1989; Conan and Carre, 1989; Savage and Deo, 1989; Trevino *et al.*, 1990; Kosson *et al.* 1994.

wall thickness and structure (Longstaff and McNab, 1987; Carre *et al.*, 1991). Reichert and Mackenzie (1982) have reported a negative correlation between starch and protein contents in peas. In addition, they showed that most of the differences in protein contents can be accounted for by starch contents. Cousin (1983) demonstrated that round-seeded pea varieties are richer in starch than the wrinkled-seeded peas.

The simple sugar profile of non-starch polysaccharides of peas, presented in Table 3 reveals that non-starch glucose is predominant. This implies that cellulose is the major component of the cell wall of peas. Reichert (1981) showed that most of the cellulose is found in the hulls, which according to Aman and Graham (1987), contain above 80% fibre and contribute about 10% of the dry matter and 60% of the cell wall material in peas. The relatively high concentrations of uronic acid and arabinose residues, which are derived from pea cotyledons (Brillouet and Carre, 1983), indicate that pectic substances are important components of pea cell wall. Other polysaccharides like hemicelluloses are also present in appreciable quantities (Aman and Graham, 1987).

Apart from cellulose, pectic substances and hemicelluloses, other components which are not polysaccharides but are associated with cell wall polysaccharides and have been found in peas are lignin, pronase-resistant cell wall protein and cell wall minerals (Reichert, 1981; Brillouet and Carre, 1983; Savage and Deo, 1989). According to Theander *et al.* (1989) all non-digestible substances, which include oligosaccharides, non-starch polysaccharides, lignin, cell wall protein and minerals make up the dietary fibre. Wright *et al.* (1984) estimated the dietary fibre of peas to be 188.0 g/kg. However, they did not include oligosaccharides, lignin, cell wall protein and minerals in this value.

### *Lipid and Mineral Content*

The lipid content of peas is very low, values ranging from 10 to 40 g/kg have been reported (Reichert and MacKenzie, 1982; Welch and Griffiths, 1984). Linoleic acid represents about half of the total lipid content (Welch and Griffiths, 1984; Savage and Deo, 1989).

Peas are almost devoid of calcium (0.3-1.4 g/kg) but well provided with phosphorus (2.2- 5.1 g/kg) (Meiners *et al.* 1976; Gad *et al.* 1982a; Savage and Deo, 1989). If available, peas could serve as a good source of phosphorus for monogastic animals. However, like other legumes, peas contain phytic acid which affects bioavailability of phosphorus (Gad *et al.* 1982a; Manan *et al.* 1987).

## ANTI-NUTRITIONAL FACTORS IN PEAS

The results of feeding trials (Moran *et al.*, 1968; Brenes *et al.*, 1993) have shown that the nutritive value of peas is lower than that predicted by its chemical composition. The presence of naturally occurring anti-nutritional factors (ANFs) which interfere with nutrient availability is responsible for suboptimal performance of animals fed peas. A number of these factors such as protease inhibitors, amylase inhibitor, tannins, haemagglutinins (lectins), phytic acid and oxalic acid have been identified.

### *Tannins*

Tannins are comprised of a diverse group of phenolic compounds. Hagerman (1988) has defined tannins as naturally occurring water-soluble phenolic compounds of

molecular weights between 500 to 3,000 capable of precipitating alkaloids, gelatins and other proteins. Those of antinutritional significance to monogastric animals and present in abundance in legumes are condensed tannins (Butler, 1988; Salunkhe *et al.*, 1990). These are tannins which do not split into sugars and phenolic carboxylic acids upon treatment with either acid or alkali (Marquardt, 1989). The nutritional consequences of the consumption of condensed tannins by monogastric animals have been reviewed by Butler (1988), Marquardt (1989) and Jansman (1993). Briefly, the negative effects of feeding high levels of condensed tannins to monogastric animals comprise of reduced feed intake - because of the astringent taste, decreased nutrient utilization (especially protein), impaired growth and reduced egg production.

The tannins in peas have been studied by a number of workers. However, it is important to note that data from these authors are difficult to correlate due to numerous analytical methods used. The range (0-3.75%) of tannin contents given in Table 4 is based on data from those authors who used the vanillin assay - an assay widely employed for the quantitative determination of condensed tannins in plants (Burns, 1971; Price *et al.*, 1978). From the table it can be seen that there is a considerable variability in tannin content of peas. The white-flowered varieties are low in tannins while the coloured-flowered varieties contain high amounts (Griffiths, 1981; Stickland, 1984; Buraczewska *et al.*, 1989). Pea tannins are confined to the seed coat.

In a series of *in vitro* experiments, Griffiths (1981) showed that testas from coloured-flowered pea varieties significantly reduced protein solubility as compared with testa from white-flowered varieties. Also, addition of extracts from the coloured-flowered

varieties significantly inhibited the activities of trypsin, chymotrypsin and α-amylase in a similar manner like coloured-flowered variety from field beans. The inhibition was found to be reversible after addition of polyvinylpyrrolidone, a tannin binding agent. The author then concluded that pea testa tannins (on a weight for weight basis) have equal potency as protein complexing agents and enzyme inhibitors as testa tannins reported for field beans. In an *in vivo* study using laying hens, Lindgren (1975) also demonstrated that peas containing higher levels of tannins had a significantly lower metabolizable energy content and crude protein digestibility than low tannin-containing peas. Since most of the tannins are contained in the testa, dehulling would reduce the tannin content of the seeds.

***Protease Inhibitors***

Protease inhibitors (trypsin and chymotrypsin inhibitors) are low molecular weight proteins capable of binding to and inactivating digestive enzymes, trypsin and chymotrypsin. The mode of action of the enzyme inhibitors is not fully understood *in vivo* but Griffiths (1984) has suggested that by inhibiting the activity of trypsin or chymotrypsin in the digestive tract, the pancreas is stimulated to synthesize and secrete more of these digestive enzymes, thereby resulting not only in a hyperactive pancreas but also in an increased demand for essential amino acids by this organ. Since pancreatic enzymes are particularly rich in the sulphur amino acids, methionine and cystine, pancreatic hypertrophy serves to divert the supply of these amino acids from the synthesis of body tissues to the synthesis of pancreatic enzymes which are irretrievably

lost by excretion (Liener, 1979). This loss in the sulphur amino acids aggravates an already critical situation with respect to pea protein which is inherently deficient in these amino acids.

The trypsin inhibiting activity (TIA) in pea seeds ranges from 0.15 to 15.9 U/mg while chymotrypsin inhibiting activity (CIA) varies between 0.74 and 10.24 U/mg (Table 4). According to Valdebouze *et al.* (1980) about 90% of the TIA in peas is located in the cotyledons and 10% in the hulls, in direct proportion with the weight distribution of these fractions in the whole seed. The TIA in peas is 5-20 fold less than that found in soybeans (Hove and King, 1979; Valdebouze *et al.*, 1980, Griffiths, 1984). However, the amounts found in some pea cultivars are adequate to cause poor growth performance in both pigs and poultry (Johns, 1987; Gatel and Grosjean, 1990).

Both genotypes and environment have been shown to influence the amounts of TIA in peas. Vaisblai (1978) found a 4 fold variation in TIA in 83 cultivars, and amongst 11 varieties grown in seven locations in Europe, Bacon *et al.* (1995) observed up to 4.5 fold variation in their TIA. It has also been shown that wrinkled-seeded varieties have less TIA than round-seeded (smooth) varieties and spring sown peas also contain less TIA than winter sown peas (Valdebouze *et al.*, 1980; Conan and Carre, 1989; Leterme *et al.*, 1990). Griffiths (1984) also reported that coloured-flowered varieties contain less TIA than white-flowered varieties.

***Haemagglutinins***

Haemagglutinins, otherwise known as lectins, are proteins which are characterized

TABLE 4. Anti-nutritional factors in peas.

| | |
|---|---|
| Amylase inhibitor (AUI/g DM) | 14.0-80.0 |
| Chymotrypsin inhibitor (CUI/mg DM) | 0.74-10.24 |
| Trypsin inhibitor (TUI/mg DM) | 0.15-15.9 |
| Haemagglutinins (HU/mg DM) | 100-400 |
| Phytic acid (g/kg DM) | 2.22-7.44 |
| Tannins (CE[1], % DM) | 0.0-3.75 |
| Oxalate (g/kg DM) | 6.67 |

[1] Catechin equivalent.
Based on data from: Hove and King, 1979; Valdebouze *et al.*, 1980; Griffiths, 1981; Davis, 1981; Gad *et al.*, 1982a; Gad *et al.*, 1982b; Griffiths, 1984; Johns, 1987; Griffiths, 1989; Savage and Deo, 1989; Leterme *et al.*, 1990; Gatel and Grosjean, 1990; Gatel, 1994; Bacon *et al.*, 1995.

by their unique ability to bind to specific sugars or glycoproteins (Liener, 1989). By binding to epithelial cells lining the small intestines, they impair intestinal permeability and nutrient transport, as well as enzymatic activities and hormonal regulation (Pusztai, 1989). This results in reduced nutrient utilization, depressed growth and ultimately death of the animal.

Information about levels of haemagglutinins in peas in relation to other legumes and their toxicity in farm animals is limited. The haemagglutinating activity (HA) reported varies from 100 to 400 units per milligram (Table 4). Valdebouze *et al.* (1980) reported that HA in peas is one-tenth of that found in raw defatted soybean meal but higher than that of field beans. In addition, the authors observed that haemagglutinins are located only in the cotyledons of pea seeds. Contreras and Tagle (1974), however, reported that HA in peas is higher than that of raw soybean meal. Betrand *et al.* (1988) fed purified pea haemagglutinin to piglets and observed no adverse effect on neither growth performance nor nutrient utilization. But Jindal *et al.* (1982) reported that haemagglutinins isolated from peas inhibited growth in rats.

### *Amylase Inhibitors*

Amylase inhibitors, proteins which interfere with pancreatic and salivary enzymes involved with carbohydrate digestion, have been found in a wide range of legume seeds (Jaffe *et al.*, 1973). Peas were found to contain negligible amounts of these compounds (Table 4) while kidney beans contained the highest amounts. Jaffe *et al.* (1973) also

reported that these inhibitors were completely inactivated at 100 C. Like trypsin inhibitors (Griffiths, 1984), it is unlikely that at temperatures below 100 C amylase inhibitors would be completely destroyed. Some amylase inhibitors have been reported to be unaffected at the temperature of boiling water (Narayana *et al.*, 1971).

***Phytate and Oxalate***

Phytate, the salt of phytic acid, is a hexa-phosphate ester of inositol which is considered to be an antinutritive factor. Its antinutritive effect lies in the fact that it readily chelates with divalent metal ions such as calcium, magnesium, zinc, and iron to form poorly soluble compounds that are not readily absorbed from intestines (Liener, 1989). Thus phytate interferes with the bioavailability of minerals from plant sources. Erdman and Forbes (1977) reported that level of 1% phytic acid in the diet interfered with mineral metabolism in rats.

In an *in vitro* study, Barre (1956) demonstrated that phytic acid inhibited the activities of proteolytic enzymes. This inhibition may result from the interaction between phytic acid and basic residues of proteins or from the chelation of calcium ions which are essential for the activities of a number of digestive enzymes (Liener, 1989). The phytic acid content of peas ranges from 2.22 to 7.44 g/kg (Table 4). The phytate content of peas is higher than that found in lentil seeds (Manan *et al.* 1987). Manan *et al.* (1987) showed that cooking resulted in a considerable reduction (82%) in the phytic acid content. However, there was no apparent relationship between the loss of phytate and the improvement in the nutritive value of peas observed in this study.

Oxalate, the salt form of oxalic acid also has the ability to form insoluble salts with divalent cations. The oxalate content of peas is reported to be 6.67 g/kg (Gad *et al* 1982b). This content is higher than that of lupin but lower than that of field beans reported by the same authors. Cooking and dehulling have been shown to reduce oxalate content (Gad *et al.* 1982b).

## THE FEEDING VALUE OF PEAS

The nutritive quality of any feedstuff does not only depend on its nutrient composition but also on digestibility and subsequent utilization of these nutrients by farm animals. Relative to pigs, much less information is available concerning the digestibility of peas in poultry. Data on available energy, protein, some amino acids and starch in peas as determined using young and adult chickens were summarized (Table 5).

The true metabolizable energy (nitrogen-corrected, $TME_n$) and apparent metabolizable energy (nitrogen-corrected, $AME_n$) contents range from 10.09 to 12.83 MJ/kg and from 8.14 to 11.63 MJ/kg, respectively. The $TME_n$ and $AME_n$ values showed considerable variability which may be attributed to genotype differences, variable starch contents, starch and protein digestibility and antinutritional factors (Lindgren, 1975; Longstaff and McNab, 1987; Conan and Carre, 1989; Carre *et al.*, 1991). Lindgren (1975) and Brenes *et al.* (1993) reported lower metabolizable energy values in tannin-containing pea varieties. Conan and Carre (1989) and Carre *et al.* (1991) compared metabolizable energy values of spring and winter peas and found that the former showed better metabolizable energy values than the latter. A mean difference of 0.45 MJ/kg of

dry matter between spring and winter peas was reported by Carre *et al.* (1991). Also coloured-flowered peas are lower in digestible energy than the white-flowered peas (Lindgren, 1975). Compared with cereal grains (NRC, 1994), canola meal (Askbrant, 1988; Simbaya, 1995) and soybean meal (Askbrant, 1988; NRC, 1994), the metabolizable energy ($TME_n$ and $AME_n$) contents of peas are comparable to that of barley, higher than that of canola meal and soybean meal but lower than that of wheat.

Like metabolizable energy, apparent digestibility of pea protein is highly variable particularly in young birds (Table 5). It is lower for coloured-flowered peas than for white-flowered peas, this difference probably results from the presence of tannins in the coloured-flowered varieties (Griffiths, 1981). Brenes *et al.* (1993) reported that pea protein digestibility could be as low as 50% in tannin-containing varieties. Among the white-flowered varieties, protein digestibility is higher for spring peas than in winter peas and it is also higher in young birds than in adults (Conan and Carre, 1989; Carre *et al.*, 1991). Data available on true amino acid digestibility are very sketchy. The digestibility of protein, lysine, methionine, cystine and threonine in peas (especially in white-flowered spring varieties) is comparable to that of soybean meal, higher than that of field beans but lower than that of lupin (Gatel, 1994).

The digestibility of pea starch (Table 5) is lower than that of the cereal grains ( over 90% digestible). Carre *et al.* (1991) did not observe any difference between young and adult birds in digestibility of pea starch. Significant differences exist among cultivars in starch digestibility (Conan and Carre, 1989). Longstaff and McNab (1987) reported a high correlation ($r^2 = 0.80$) between starch digestibility and $TME_n$ of peas, indicating

TABLE 5. Metabolizable energy content (MJ/kg DM), apparent protein, true amino acid and starch digestibility (%) values of peas.

| | |
|---|---|
| True metabolizable energy (nitrogen-corrected)[a] | 10.09-12.83 |
| Apparent metabolizable energy (nitrogen-corrected)[b] | 8.14-11.63 |
| Apparent protein digestibility | 70.3-78.7[a] |
| | 50.0-84.2[b] |
| True amino acid digestibility[a] | |
| Lysine | 86.9 |
| Methionine | 88.6 |
| Cystine | 77.7 |
| Threonine | 87.7 |
| Starch digestibility | 81.2-87.0[a] |
| | 78.7-87.0[b] |

[a] Determined using adult cockerels.
[b] Determined using young chicks.
Based on data from: Lindgren, 1975; Askbrant and Hakansson, 1984; Sibbald, 1986; Longstaff and McNab, 1987; Askbrant, 1988; Conan and Carre, 1989; Carre *et al.*, 1991; Brenes *et al.*, 1993; Racz, 1994; Gatel, 1994.

that any increase in starch digestion would correspond to an increase in $TME_n$.

Unlike in hog feeding, peas remain an under-utilized feed ingredient in poultry feeding. They are considered as a moderate source of protein and energy in poultry diets. The prime nutritional advantages of peas are relatively high lysine content and favourable essential amino acid balance. However, in common with many other leguminous seed crops, low content of sulphur amino acids (methionine and cystine) and the presence and the variability of antinutritional factors reduce the feeding value of peas for poultry.

***Use of Peas in Broiler Diets***

A number of studies have looked at the suitability of peas as protein and energy supplements in broiler diets. The limits of incorporation of peas in the diets without any detrimental effects on performance characteristics varied from author to author, most probably because of differences in experimental methodology as well as differences in the quality of peas used. Lettner *et al.* (1986) showed that up to 30% of the diet of broilers could be made up from peas without any negative effects on production except that linoleic acid content of the body fat decreased with increasing peas in the diet. Moran *et al.* (1968), however, encountered significant depression in chick growth and feed utilization when peas were fed at 35%. Brenes *et al.* (1989) fed 80% peas under commercial production conditions and found that birds fed peas performed better than those offered the comparable soybean meal control diet. The birds on the control diet had lower feed consumption which the authors attributed to the physical nature of the diets. More recently, the same authors (Brenes *et al.* 1993) also demonstrated that satisfactory

growth performance of chicks could be obtained at 48% peas in the diet. Conversely, several studies (Kienholz *et al.* 1962; Moran *et al.* 1968; Goatcher and McGinnis, 1972; Johns 1987) have shown that incorporation of peas in broiler diets at 40% and above resulted in a significant reduction in growth rate, feed consumption and feed utilization to an extent in which neither methionine supplementation nor any form of heat treatments could counteract the effects.

Methionine supplementation, autoclaving and pelleting had positive effects on the nutritive value of peas (Moran *et al.* 1968; Johns, 1987), however, their effectiveness would depend on the dietary inclusion level of peas.

***Use of Peas in Layer and Breeder Diets***

Few experiments have been carried out to establish the inclusion level of peas in layer diets. As with the broilers, results published from these experiments are controversial. Moran *et al.* (1968) fed pelleted or unpelleted diets containing 15 or 30% peas supplemented with methionine and found no adverse effect on egg production or egg weight. However, feed conversion efficiency of birds on all pea diets was significantly lower than those fed the maize-soybean control diet. Similarly Lindgren (1975) and Askbrant and Hakansson (1984) incorporated peas into laying hen diets at 15 or 30% and observed that egg production, feed consumption and body weight were not affected by these dietary inclusions. Castanon and Perez-Lanzac (1990) used up to 50% peas in their study without any adverse effects on egg production, feed intake or feed conversion. In fact, egg weight increased slightly with increasing levels of peas in the diet. Another

recent study by Ivusic *et al.* (1994) also demonstrated that peas could be incorporated into layer diets at high levels of inclusion. Only birds fed 59% peas laid eggs with thinner egg shells than those fed the corn-soybean control diet and the decreased shell quality was observed in the experiment after 38 weeks of age. It is important to mention that these authors used a yellow pea cultivar, Miranda, which is probably low in antinutrient contents.

Contrary to the above findings, Davidson (1977) reported that when laying hen diets contained 17 and 37% peas, egg production was 20 and 45% lower, respectively, than the fishmeal control diet. Either the addition of methionine or heat processing the peas significantly improved output but was not adequate to restore full egg production. In 1980, the same author recorded a decrease of about 47% in egg production at 37.5% peas in the diet. In this study he found that heat processing coupled with the addition of methionine output was improved to give the same production-rate as the fishmeal control diet. A year later, Davidson *et al.* (1981) confirmed the earlier experiments that high inclusion levels of peas in layer diets had detrimental effects on egg production. At 41.5% inclusion level, they encountered a depression in egg production as well as in daily feed intake and body weight. However, egg weight was not affected. It is not yet clear what level of peas can be included in layer diets without adversely affecting production performance.

There is little in the literature concerned with the value of the field peas in breeder diets. Rakphongphairoj and Savage (1988) using a yellow pea variety, Miranda, incorporated 14 and 61% peas in corn-based diets formulated to contain 7 and 16% crude

protein (CP), respectively, and fed to broiler breeder males on an *ad libitum* and a restricted basis, respectively. The performance of the birds were compared with their respective corn-soy diets. Broiler breeder males fed the 7% CP corn-soy diet produced more semen than the 7% CP corn-pea diet (containing 14% peas), however, this difference was not observed with birds fed 16% CP corn-soy and 16% CP corn-pea diet (containing 61% peas). Total testicular weight, fertility and hatchability of fertile eggs were not affected by dietary inclusion of peas. These results were corroborated by Bootwalla *et al.* (1988) who reported that feeding broiler breeder males diets containing yellow peas (Var. Miranda) did not have any detrimental effects on semen quality, fertilizing capability and subsequent hatchability of fertile eggs. Because tannins inhibit nucleic acid and protein synthesis at the cellular level (Singleton, 1981), feeding high tannin peas to broiler breeder males may affect semen production and quality. However, there is no literature documentation for this assumption.

## IMPROVEMENTS OF THE NUTRITIVE VALUE OF PEAS

### *Breeding and Genetic Manipulation*

The nutritive quality of peas could be improved through the use of appropriate breeding strategies. Breeding strategies that involve reducing or eliminating toxic substances, like protease inhibitors, lectins and polyphenols and improving the content and quality of the pea protein (that is the proportion of protein in the total dry matter and its amino acid composition) would be useful.

Considerable variations for seed protein content among pea genotypes which might

form the basis for further selection and production of improved varieties with high protein content have been reported. Among 225 pea lines, Matthews and Arthur (1985) found a three-fold variation in their protein contents. In addition, they also observed that about 12% of these lines had protein levels in excess of 27% which could be regarded as high-protein lines and could be potential parents for the breeding of high protein varieties. Unlike in many cereal species where yield and protein content are negatively correlated, the relationship between yield and protein content of peas is characterized by either a weak or no correlation (Ali-Khan and Youngs, 1973; Pandey and Gritton, 1976; Cousin, 1983) which makes it possible to improve seed protein content without any negative effect on yield. Similarly, there is no correlation between seed size and seed protein content (Ali-Khan and Youngs, 1973; Cousin, 1983). Since seed size is an important component of yield, therefore, it should be possible to select and to develop pea lines which simultaneously show high yield and high protein.

The efficiency of selection for improved protein content may be hampered by large and significant environmental effects. Karjalainen and Hovinen (1981) reported that one-third of the variation in protein content in their study was accounted for by climatic factors. This observation was confirmed by Matthews and Arthur (1985) who reported that genetic variability is almost completely masked by unpredictable environmental variables acting either within the agricultural environment or within the plant itself. The implication of this is that any breeding programme aimed at increasing protein content which does not account for environmental variation could be a frustrating and unrewarding exercise (Matthews and Arthur,1985). Apart from environmental influence,

legume seed protein in general is of low to medium heritability (Bliss and Hall, 1977) because it is mainly controlled by recessive factors (Cousin *et al.*, 1985). However, in spite of all these difficulties, many attempts have been made to improve protein content in peas through selection. In Hungary selection within a cultivar has resulted in 2-3 percent units increase in protein content without changing important characteristics of the cultivar (Kurnick *et al.*, 1970). Pandey and Gritton (1976) also reported that percentage of protein was increased by 5.9 percent units of the population mean through selection.

The protein of pea seeds is limited in its nutritional suitability for monogastric animals by its low content of methionine. It has been demonstrated that the concentration of methionine is negatively correlated with protein level in peas (Evans and Boulter, 1980). Therefore, above a certain protein level, additional protein that may be synthesized and stored due to selection for high protein would result in lower concentration of methionine which represents poorer protein quality. Methionine content therefore should play an important role in any breeding strategies for protein quality improvement. Smartt *et al.* (1975) have discussed a number of ways to improve the protein quality of grain legumes by breeding and suggested that such a process should proceed by selection for genotypes which produce maximal amounts of those proteins with desirable methionine and cystine contents.

The concentrations of methionine and cystine are a function of the proportion of vicilin to legumin (two major storage globulins) in pea protein. Vicilin is usually the major of the two proteins in most pea genotypes and is very deficient in methionine and cystine (Croy *et al.* 1980; Casey *et al.* 1982). On the other hand, legumin is high in

methionine and cystine (Casey and Short, 1981). Therefore any increase in the proportion of legumin relative to vicilin would be an important objective in the breeding of peas with protein of increased methionine and cystine contents. Genes which significantly affect the legumin deposition should be identified and used to improve the methionine and cystine contents of peas.

Published data on trypsin inhibiting activity (TIA) in peas revealed considerable variations. Vaisblai (1978) found a 3.9-fold variation in 83 cultivars, and amongst 63 pea genotypes, Domoney and Welham (1992) reported more than a 10-fold variation. Many workers have also linked observed variations in TIA to particular phenotypic characteristics. For example, Valdebouze *et al.* (1980) observed that round-seeded peas are higher in TIA than the wrinkled seeded varieties. Peas with coloured-flowers are reported to have, generally, lower TIA than white-flowered varieties (Pisulewski *et al.* 1983). Also winter sown peas are claimed to have higher TIA than peas sown in the spring (Valdebouze *et al.* 1980). More recently Bacon *et al* (1995) reported the influence of environmental factors on TIA and they also identified a number of pea lines that showed low TIA across a range of environments. All these observations provide incentives for pea breeders to develop varieties, through selection, free of or low in TIA which may have enhanced nutritional properties.

Tannins in peas are restricted to the seed coat of the coloured-flowered varieties (Griffiths, 1981). In relation to breeding programmes designed to maximize nutritive value of peas, it would appear that the tannin content of the selections made should be minimized by selecting for white-flowered tannin-free varieties. However, in removing

the ANFs through breeding, one has to consider the implications on agronomic characteristics like resistance to adverse weather conditions and resistance to diseases and predators.

***Dehulling and Fractionation of Pea Seeds***

Peas contain ANFs which are distributed in seed fractions. The pea hulls contain more polyphenols and dietary fibre than the pea cotyledons while the protease inhibitor content of the pea cotyledons is about 9 times higher than that of the pea hulls (Valdebouze *et al.* 1980). Processing methods based on separation of pea seeds into fractions with high and low levels of ANFs offer a promise in improving the nutritive value of peas. Dehulling enriched the nutrient concentration of peas. The crude protein, amino acid and starch contents increased while the fibre content decreased (Longstaff and McNab, 1987; Savage and Deo, 1989; Brenes *et al.* 1993) and the polyphenolic contents decreased substantially (Griffiths, 1981; Brenes *et al.* 1993) by dehulling.

Dehulling increased true metabolizable energy (TMEn) of ground peas from 9.91 to 12.39 MJ/kg as determined with adult cockerels (Longstaff and McNab, 1987). Starch digestibility also increased from 75.6 to 93.1%. Similarly, Brenes *et al.* (1993) reported that dehulling increased apparent metabolizable energy (AMEn) and apparent protein digestibility (APD) of tannin-free and tannin-containing peas in leghorn chicks. In their study, the AMEn and APD of the tannin-containing peas increased by 30 and 70% respectively, whereas the AMEn and APD of the tannin-free peas increased by 4 and 11% respectively.

Although dehulling increases nutrient concentrations, protein and starch digestibilities and the metabolizable energy value, feeding low tannin dehulled peas to chicks only produced a marginal increase in weight gain and feed utilization (Brenes *et al.* 1993). However, these authors reported a substantial improvement in performance of chicks fed dehulled tannin-containing peas. The lack of significant response in chicks fed dehulled peas in some cases may be related to the increased protease inhibitor content. As mentioned earlier, protease inhibitors are present in cotyledons at much higher concentrations than in the hulls.

With the application of impact-milling and air classification technology, whole peas can be fractionated into pea hulls, pea protein concentrate and pea starch (Vose *et al.* 1976) to enhance their utilization in the food industry. Pea hulls are used for making high fibre white bread and the starch fraction has application in adhesives and carbonless paper. Pea protein concentrate has limited application in the food industry but it has been used as an extender in the production of ground beef (Vaisey *et al.* 1975).

The most unfortunate aspect of impact-milling and air-classification is that they concentrate ANFs into the starch and protein fractions. Because most ANFs have relatively low molecular weights and are proteinaceous, they tend to separate into the protein fraction. From Table 6 it can seen that the protein fraction contains more ANFs than both the pea flour and the starch fraction.

Because of its protein content and amino acid profile, it was initially thought that pea protein concentrate would be the most valuable product of the milling and fractionation processes, especially in human and monogastric animal nutrition. However,

its potential has not been fully realized. The crude protein content (55-67%) of protein concentrate is comparable to that of meat meal, fish meal and higher than that of soybean meal and canola meal (Keith, 1977; Sosulski and Youngs, 1979; Fleming and Reichert, 1983; Bhatty and Christison, 1984). The amino acid profile is quite different from that of the pea flour especially methionine and tryptophan (Vose *et al.* 1976; Carnovale and Cappelloni, 1983) and is similar to the amino acid profile of soybean meal, with the exception of methionine (NRC, 1994).

The digestibility of pea protein concentrate has been studied in a number of livestock species. Bell and Youngs (1970) reported the protein digestibility of pea protein concentrate as 83.5% in wealing mice. This was later corroborated by Bhatty and Christison (1984) who reported a digestibility value of 85.4%. When pea protein concentrate provided 50% of total protein in milk replacers for calves protein digestibility value was 50% at 7 to 9 days of age and 79% at 22 to 24 days of age (Bell *et al.* 1974). Mbugi *et al.* (1989) also reported a protein digestibility value of 78.7% for days 18 to 28 of age when pea protein concentrate supplied 60% of total protein in milk replacers. There is no literature documentation on the nutritional value of pea protein concentrate in poultry diets.

***Heat Treatments***

The potential for improving the nutritive value of peas with the application of heat treatments has been recognized for over half a century. In an early study, Woods *et al.* (1943) used autoclaving and baking to treat raw peas for rat diets and since then, other

TABLE 6. Distribution of various anti-nutritional factors (ANFs) in pea flour and fractions after air-classification.

| ANF | Flour | Protein | Starch |
|---|---|---|---|
| Trypsin inhibitor (TUI/mg) | 7.61 | 23.35 | 1.43 |
| Haemagglutinins (HU/mg) | 15.06 | 39.99 | 1.90 |
| Saponin activity (HA/g) | 0 | 0 | 0 |
| Phytic acid (mg/g) | 7.44 | 18.88 | 1.82 |
| Tannins (CE[1], %) | 0.25 | 0.53 | 0.12 |
| Insoluble dietary fibre (%) | 3.20 | 6.79 | 1.81 |

[1] CE = catechin equivalent.
Based on data from: Davis, 1981; Elkowicz and Sosulski, 1982.

heat treatments like flaking, extrusion, steam pelleting, dry heating and micronization have been tested. Generally, published information showed that heat processing is an effective method of improving the nutritive value of peas for monogastric animals, especially poultry. The mechanism through which nutrients are better available after heat treatments may result from an increased accessibility of these nutrients to enzyme attack or from the inactivation of proteinaceous ANFs, primarily trypsin inhibitors and lectins (Poel, 1990). Low accessibility of pea protein and starch to enzyme attack caused by strong cellular cohesion in pea cotyledons has been suggested to be a major factor responsible for their poor digestibilities and the overall performance of birds fed raw peas (Carre *et al.*, 1991). Thermal treatments could be used to break up cell walls to facilitate nutrient-enzyme contacts. Because of the protein nature of trypsin inhibitors and lectins, they can be destroyed by heat processing. According to Rackis *et al.* (1986), protease inhibitors require their structural integrity in order to inactivate proteolytic enzymes by complex formation and heat denaturation could disrupt this structural integrity. The effectiveness of heat treatments on the ANFs and on the nutritional value of legume seeds is a function of process temperature, duration of heating, particle size, moisture content and variety (Liener, 1983; Poel, 1989). Various combinations of temperature and time, intensity of pressure and moisture at appropriate stages are being used depending on the heat treatment method chosen.

A summary of the overall effects of heat treatments on trypsin inhibiting activity (TIA) and haemagglutinating (lectin) activity (HA) in peas are presented in Table 7. It should be noted that data from different authors are difficult to correlate due to

incomplete information on heating conditions. The data reveal that a considerable reduction in TIA and HA can be achieved by heat treatments. Loss of TIA and HA is almost complete when raw peas were subjected to autoclaving, extrusion or micronization processing (Tannous and Ullah, 1969; Zuilichen and Poel, 1989; Poel *et al.*, 1992). However, the amount of heat needed to completely inactivate these ANFs would depend on the type of peas. While extrusion at 105 C slightly reduced TIA and had no effect on HA in wrinkled-seeded peas but extrusion at the same temperature fully inactivated TIA and most of HA in round-seeded peas (Poel *et al.*, 1992). For wrinkled-seeded peas, the authors found that temperatures above 125 C would be necessary to inactivate TIA and HA totally. The potential of steam pelleting as a means of reducing TIA seems very low. The amount of TIA reduced by sinlge steam pelleting at temperatures below 100 C varied between 0 and 47% (Carre *et al.*, 1987; Grosjean and Gatel, 1989; Carre *et al.*, 1991) but with double steam pelleting up to 50% reduction could be achieved (Carre *et al.*, 1987). The reason for this low reduction could be due to the stability of trypsin inhibitors at lower temperatures. Griffiths (1984) showed that trypsin and chymotrypsin inhibitors were stable at temperatures below 100 C.

Heat treatments significantly improve protein and starch digestibilities (Table 8). Chicks seem more responsive to treatment than adult cockerels. For autoclaving and extrusion, the improvements could be attributed, in part, to the reduction in the ANFs. However, for steam pelleting, the effect might largely be due to the breakdown of the cell walls of pea cotyledons, which allows the accessibility of nutrients to digestive enzymes, rather than to the reduction of ANFs (Carre *et al.*, 1991). Moran (1982) pointed out that

TABLE 7. Effects of heat treatments on the inactivation (%) of trypsin inhibiting activity (TIA) and Haemagglutinating (lectin) activity (HA) in peas.

| Heat treatment | | | Reduction of TIA | Reduction of HA | References |
|---|---|---|---|---|---|
| Process | Heating conditions | | | | |
| | Temp (°C) | Time | | | |
| Autoclaving | 121 | 30 min | - | 95 | Muelenaere, 1965 |
| Autoclaving | 121 | 5 min | 100 | 100 | Tannous and Ullah, 1969 |
| Dry heating | 60-100 | 24 hr | 0-26 | - | Griffiths, 1984 |
| Single steam pelleting | 88 | - | 47 | - | Carre *et al.*, 1987 |
| Double steam pelleting | 88 then 75 | - | 51 | - | Carre *et al.*, 1987 |
| Autoclaving | 130 | 3 min | 40-86 | - | Conan and Carre, 1989 |
| Steam Flaking | - | - | 90 | - | Focant *et al.* 1989 |
| Micronization | - | - | 90 | - | Focant *et al.*, 1989 |
| Extrusion | - | - | 96 | - | Focant *et al.*, 1989 |
| Extrusion | 105-135 | - | 100 | 88-100 | Zuilichen and Poel, 1989 |
| Micronization | 124 | 65 sec | 100 | 100 | Zuilichen and Poel, 1989 |
| Extrusion | - | - | 100 | - | Grosjean and Gatel, 1989 |
| Single steam pelleting | 80 | - | 30 | - | Grosjean and Gatel, 1989 |
| Single steam pelleting | 81 | - | 0-14 | - | Carre *et al.* 1991 |
| Extrusion | 105-135 | - | 100[1] | 87-100 | Poel *et al.*, 1992 |
| Extrusion | 105-135 | - | 46-90[2] | 0-100 | Poel *et al* 1992 |

[1] Values obtained when round-seeded peas were extruded.
[2] values obtained when wrinkled-seeded peas were extruded.

the difficulties encountered by fowl in digesting legume starch could be alleviated by heat treatments that initiate granule gelatinization. Gelatinized or disrupted starch is more rapidly degraded by enzymes than raw starch (McNeill *et al.*, 1975). Also, Nordheim and Coon (1984) showed that some form of heat treatment is required to alter the three-dimensional structure of plant proteins to allow the protein to be more susceptible to enzyme hydrolysis.

In the experiment carried out by Brenes *et al.* (1993), autoclaving had more effect on protein digestibility of the tannin-containing pea cultivar (73 vs 81%) than tannin-free cultivar (80 vs 83%). However, the authors did not show if autoclaving reduced assayable tannin as reported for faba beans (Marquardt and Ward, 1979). The improvements in metabolizable energy values could be explained by the improvements in protein and starch digestibilities (Longstaff and McNab, 1987; Conan and Carre, 1989).

Heat treatment is a critical factor influencing the biological value of amino acids. Excessive heating with regards to temperature and/or exposure time to heat can adversely affect the availability of lysine, methionine and cystine (Bjarnason and Carpenter, 1970) in intact pea proteins. Overheating of protein may decrease digestibility and cause a slower release of amino acids from the protein (de Wet, 1982). Accurate control of heating conditions are therefore necessary to the processing of pea protein with maximum nutritional value.

### *Use of Exogenous Enzymes*

Another way in improving the nutritive value of peas is through the use of

TABLE 8. Effects of heat treatment of peas on nitrogen-corrected metabolizable energy ($ME_n$), apparent protein digestibility (APD) and starch digestibility in poultry.

| Heat treatment | | | Animal | $ME_n$ (MJ/kg)[1,2] | APD (%)[1] | Starch Dig. (%)[1] | References |
|---|---|---|---|---|---|---|---|
| Process | Heating conditions | | | | | | |
| | Temp (°C) | Time | | | | | |
| Autoclaving | 121 | 15 min | Chicks | 10.4 vs 11.5 | 38 vs 45[3] | - | Moran *et al.*, 1968 |
| Single steam pelleting | 90 | - | Chicks | 10.4 vs 11.3 | - | - | Moran *et al.*, 1968 |
| Flaking | 160 | - | Adult cockerels | - | 78 vs 82 | - | Huyghebaert *et al.*, 1979 |
| Extrusion | 140 | - | Adult cockerels | - | 85 vs 86 | - | Huyghebaert *et al.*, 1979 |
| Single steam pelleting | 60 | - | Adult cockerels | - | 85 vs 87 | - | Huyghebaert *et al.*, 1979 |
| Single steam pelleting | 88 | - | Adult cockerels | 12.8 vs 13.0 | 81 vs 83 | 92 vs 97 | Carre *et al.*, 1987 |
| Double steam pelleting | 88 then 75 | - | Adult cockerels | 12.8 vs 13.3 | 81 vs 83 | 92 vs 97 | Carre *et al.*, 1987 |
| Autoclaving | 121 | 30 min | Adult cockerels | 11.3 vs 11.3, $TME_n$ | - | 88 vs 91 | Longstaff and McNab, 1987 |
| Autoclaving | 130 | 3 min | Chicks | 10.4 vs 12.3 | 75 vs 86 | 80 vs 94 | Conan and Carre, 1989 |
| Single steam pelleting | 81 | - | Chicks<br>Adult cockerels | 10.9 vs 12.5<br>11.3 vs 12.3 | 76 vs 81<br>75 vs 72 | 81 vs 96<br>84 vs 96 | Carre *et al.*, 1991 |
| Autoclaving | 121 | 20 min | Chicks | 9.2 vs 9.1[4]<br>8.1 vs 9.9[5] | 80 vs 83[4]<br>73 vs 81[5] | - | Brenes *et al.*, 1993 |

[1] Untreated vs heat treated peas.
[2] Unless otherwise indicated values given are apparent metabolizable energy ($AME_n$).
[3] Net protein utilization.
[4] Tannin-free cultivar; [5] Tannin-containing cultivar.

exogenous enzyme supplements. Enzymes are biocatalysts produced by living cells to bring about specific biochemical reactions generally forming parts of the metabolic process of the cells (Sears, 1994). Enzymes used as feed additive are produced by fermenting microorganisms, like fungi and bacteria, on selected substrates. Every enzyme has its own distinct properties, like specific activity, substrate affinity, stability, pH and temperature sensitivity. The benefit of using enzymes in poultry diets, especially barley-based diets, has been recognized for over three decades (Fry *et al.* 1958). However, commercial use of exogenous enzyme supplements in poultry diets is relatively new.

Enzymes have been used to degrade different structural carbohydrates found in cereals and legumes particularly those that are not digested by avian and mammalian enzymes, and those that are highly viscous and have high water-binding capacity (Marquardt, 1994). The structural carbohydrates in cereals mainly consist of arabinoxylans (pentosans), β-glucans and cellulose. In legumes they are mainly pectins, oligosaccharides of the raffinose family and cellulose. Wheat, rye and triticale are cereals with large amounts of arabinoxylans while barley and oats mainly contain large amounts of β-glucans. Leguminous seeds, like soybeans, peas, lupins and field beans contain pectins and oligosaccharides like verbascose, stachyose and raffinose. These polysaccharides exhibit antinutritional effects.

The mechanism whereby the polysaccharides of cereals affect their nutritional value is not fully understood. Perhaps the simplest and best documented mode of action is that the arabinoxylans and/or β-glucans can form viscous materials which are believed to act as barriers to diffusion of nutrients within the gut lumen, reducing access of

digestive enzymes to their substrates, limiting rate and amount of nutrient absorption (Pawlik *et al.* 1990; Campbell and Bedford, 1992; Guenter, 1993). The common observations are that the digestibility values of fat, starch, protein and metabolizable energy of cereals that contain arabinoxylans and/or β-glucans decreased as their dietary levels increased, especially in broiler feeds (Aman and Hesselman, 1984; Annison and Johnson, 1989; Al-Athari and Guenter, 1989; Rotter *et al.* 1990; Friesen *et al.* 1992).

Numerous studies have shown a beneficial response to dietary enzyme supplementation of barley, oats, wheat, rye and triticale diets, with improvement noted for digestibility of nutrients and metabolizable energy, growth rate as well as feed utilization. Pentosanase (xylanase) supplementation of wheat and rye diets improved apparent metabolizable energy, apparent protein and lipid digestibilities and the overall performance of broiler chickens (Friesen *et al.* 1992; Marquardt *et al.* 1994). Using β-glucanase, Rotter *et al.* (1989) showed that broilers fed a high viscosity barley were able to sustain growth at an equivalent or superior to those fed a wheat diet. In addition to growth rate and feed utilization, the amounts of sticky droppings (which cause vent pasting) were drastically reduced. The same authors (Rotter *et al.* 1990) also showed an improvement in energy and nitrogen utilization of barley diets supplemented with enzyme. The effect of enzyme is achieved by the cleavage of relatively few linkages, albeit enough to result in the loss of the viscous-forming properties of the soluble arabinoxylans and β-glucans in cereals (Slominski *et al.* 1993).

The proven value of the addition of enzymes to cereal-based diets has stimulated interest in the application of enzymes to legume-based diets. As mentioned earlier, legume

seed crops are high in pectic substances and oligosaccharides which are also considered to exhibit antinutritional properties when fed to chickens (Cleophas *et al.* 1995). Coon *et al.* (1990) reported considerable improvements (20%) in the true metabolizable energy (TME) content of soybean meal fed to adult roosters with oligosaccharide removal by ethanol-extraction. In addition dry matter digestibility increased by 13.4 percent units.

Unlike in cereal diets, reports on the use of enzymes in legume containing diets are very few. Supplementing diets based on soybean meal with various enzyme preparations (amylases, proteases and lipases) produced only small and in some cases no improvement in performance of chicks (Anderson and Warnick, 1964). Longstaff and McNab (1987) found no significant improvement in the TME value of peas by feeding a commercial cellulase preparation, however, a slight increase in fibre digestion was observed. Also starch digestibility was not affected by augmenting the birds' amylases. Castanon and Marquardt (1989) supplemented diets containing field beans (*Vicia faba*) with cellulase, protease and a combination of protease plus cellulase and found small improvements in weight gain and feed utilization. More recently, Brenes *et al.* (1993) examined the effects of supplementing tannin-free and tannin-containing pea diets with a multi-enzyme complex (containing hemicellulase, pectinase and β-glucanase), Bio-Feed Pro® (proteolytic enzyme) and cellulase on performance of chicks. While they recorded no improvement in both weight gain and feed utilization by using these ezymes alone or in combination in the tannin-free pea diets, they observed significant improvement in feed utilization in the tannin-containing pea diets but weight gain was not affected. The same authors (Brenes *et al.* 1993) added a combination of Energex plus Bio-Feed Pro® plus

Novozyme® (α-galactosidase) to a diet containing lupins and obtained 18 and 10% improvements in weight gain and feed utilization, respectively, of broiler chicks. In the same study, when the optimum concentration of enzymes was determined in lupin diets, addition of 3% Bio-Feed Pro® alone increased weight gain by 24% and the feed utilization by 11%.

According to Chesson (1993) enzyme supplementation must be able to demonstrate a sufficiently substantial improvement in feed conversion or the quality of the product to cover the cost of supplementation and to provide an increased margin for the producers. While this increased margin has been realized with enzyme supplementation of barley, savings on formulation as a result of enzyme supplementation have not been justified in leguminous seed crops.

# CHAPTER THREE

## QUALITY CHARACTERISTICS OF PEAS

There are two manuscripts in this chapter, manuscripts 1 & 2. In manuscript 1, the chemical composition and the nutrient digestibility of peas were discussed. Manuscript 2 addresses the influence of location, nitrogen fertilization and seed inoculation on seed protein content and AA composition.

# MANUSCRIPT 1

## FIELD PEAS: CHEMICAL COMPOSITION AND NUTRIENT DIGESTIBILITY IN POULTRY.

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**ABSTRACT.** Twelve cultivars of peas (yellow-, green- and brown-seeded) were evaluated for chemical composition and digestibility in chicken. The evaluation involved the analyses for protein, amino acids (AAs), fat, starch, dietary fibre, ash, calcium, phosphorus and tannins. True metabolizable energy (nitrogen corrected ($TME_n$) and uncorrected (TME)) and true AA bioavailability values were also determined with adult cockerels. The cultivars showed a wide range of protein (207.5-264.0 g/kg) and starch (385.3-436.8 g/kg) contents which were not related to the seed coat colours. The concentrations of several AAs varied among the cultivars. With the exception of arginine, on protein basis, the concentrations of all other essential AAs decreased as protein levels increased. The dietary fibre contents varied between 190.7 to 223.1 g/kg and the values were slightly higher in the brown-seeded cultivars. The brown-seeded cultivars contained appreciable quantities of tannins while the yellow- and green-seeded cultivars were devoid of tannins. The cultivars were almost devoid of fat and calcium but relatively high in phosphorus. Starch and dietary fibre were negatively correlated (r = -0.78 and -0.46, respectively) with protein content and accounted for the greatest difference in protein content. Out of 10 essential AAs including cystine, only arginine had a positive correlation (r = 0.79) with protein content. The TME values ranged from 11.6 to 13.3 MJ/kg while the $TME_n$ values ranged from 11.0 to 12.9 MJ/kg. The mean availabilities of different AAs ranged from a high of 89.6 to 75.9% with total sulphur AAs (cystine and methionine) having the lowest value and glutamic acid having the highest value. There was a trend towards lower AA bioavailability values in the brown-seeded cultivars. It can be concluded that these cultivars varied in chemical compositions, metabolizable energy contents and bioavailabilities of AAs.

**Key words**. Field peas, composition, digestibility, chicken.

# INTRODUCTION

Field peas (*Pisum sativum* L.) can provide an excellent source of dietary protein for animal feeds. They are widely used in swine diets particularly in European countries (see review by Gatel and Grosjean, 1990 ).

In recent years, the quantity of peas produced in Western Canada has greatly increased. Total land area seeded to peas was 73,600 ha in 1984 whereas in 1994 over 640,000 ha was devoted to pea production (Slinkard, 1994), representing a 7-8 fold increase in one decade. This large increase in production has resulted in a tremendous increase in the number of registered pea cultivars. Among the newly registered cultivars are Fluo, Montana, Baroness, Highlight, Titan, Sirius, Radley, Impala, Carman, Trump, and Express. Many of these cultivars were developed in Europe.

In Western Canada, poultry diets are based on soybean meal to supply the protein requirements. Soybeans are not locally produced but are imported. Although canola meal is gaining some acceptance in the feed industry, its utilization is limited because of high dietary fibre and low energy contents (Bell, 1993). Peas are likely to become an increasingly important home-grown protein source for poultry and swine but at present their use is very limited. Lack of adequate nutritional information may, in part, be responsible for the low utilization.

Analytical data on crude protein (CP), amino acids (AAs), minerals and fibre contents, as well as energy digestibility value for peas in general can be found in standard tables (NRC, 1994). However, studies (Holt and Sosulski, 1979; Monti, 1983; Conan and Carre, 1989; Igbasan and Guenter, 1996a) have shown that major differences

exist in composition and digestibility among pea cultivars. Factors including location, variety and prevailing growing conditions have been reported to influence the composition and nutritive quality of peas (Ali-Khan and Youngs, 1973; Holt and Sosulski, 1979; Igbasan *et al.* 1996). Nutritionists and feed manufacturers need data specific to pea cultivars available in a locality to enable them to formulate balanced diets containing peas. And plant breeders need such data in order to develop better varieties.

The objective of this investigation was to provide detailed chemical composition of pea cultivars and to determine the energy and amino acid digestibilities in poultry.

## MATERIALS AND METHODS

### *Pea Samples*

Twelve cultivars were evaluated in this study (Table 9). Seed samples from 8 of these cultivars were kindly supplied by Dr. T.D. Warkentin, Agriculture and Agri-Food, Canada Research Station, Morden, Manitoba. The remaining 4 cultivars were obtained from pea growers located in Manitoba, Canada. Each pea sample collected was subsampled for chemical analyses. About 1 kg seeds from each cultivar were manually dehulled after soaking in cold water for 4 hr. After dehulling, cotyledons and hulls were air-dried at room temperature for 48 hr before weighing. All samples were ground to pass through a 1 mm sieve.

### *Analytical Methods*

Samples were analyzed for dry matter (DM), CP, AAs, starch, dietary fibre, fat

TABLE 9. Anatomical features of seeds derived from the pea cultivars.

| Cultivars | Weight, g/1000 seeds | Coat colour | Hull, %[1] |
|---|---|---|---|
| Express, 1987[2] | 229.2 | Y | 9.1 |
| Highlight, 1993 | 196.6 | Y | 9.1 |
| Baroness, 1993 | 259.2 | Y | 8.2 |
| Titan, 1985 | 199.0 | Y | 8.7 |
| Fluo, 1993 | 291.0 | Y | 8.5 |
| Montana, 1993 | 271.6 | Y | 9.5 |
| Impala, 1993 | 265.2 | Y | 8.0 |
| Tara, 1978 | 170.2 | Y | 8.1 |
| Radley, 1990 | 185.0 | G | 9.5 |
| Trump, 1990 | 198.6 | G | 9.5 |
| Carman, 1992 | 243.8 | B | 12.0 |
| Sirius, 1989 | 188.8 | B | 11.6 |

[1] Percent of hull in dry seed.
[2] Year in which each cultivar was registered for production (T. D. Warkentin 1993; personal communication), Agriculture and Agri-Food, Canada Research Station, Morden, Manitoba.
Y = Yellow; G = Green; B = Brown.

(ether extract), ash, calcium, phosphorus and tannin.

Dry matter, ash, ether extract, calcium and phosphorus were determined by standard methods of analysis (Association of Official Analytical Chemists, AOAC, 1990). Nitrogen was determined by Kjeldahl procedure (AOAC, 1990) and converted to protein content using a 6.25 conversion factor.

The method described by Slominski *et al.* (1994) was used to estimate the dietary fibre content of peas. In this method, dietary fibre content was determined as the sum of neutral detergent fibre (NDF) and detergent-soluble non-starch polysaccharides (NSP). The NDF component was determined using a refluxing apparatus[1] according to a procedure outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with the addition of α-amylase enzyme (Termamyl)[2]. The NSP contents of pea samples and NDF residues were determined by gas liquid chromatography using the procedure described by Englyst and Cummings (1984) with some modifications (Slominski and Campbell, 1990). Detergent-soluble NSP was calculated by difference (sample NSP minus NSP present in NDF residue). Cell wall protein and ash contents present in NDF residues were also determined and the value for lignin and associated polyphenols was calculated as [NDF - (NSP + protein + ash)]. Starch was determined using the NSP procedure of Englyst and Cummings (1984) and calculated by difference between the total glucose content and the glucose present in the NSP residue following

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[1]Laboratory Construction Col. Kansas City, MO.

[2]Novo Nordisk A/S, Bagsvaerd, Denmark.

hydrolysis with α-amylase and pullulonase enzymes.

Tannin content was measured by the method of Burns (1971) as modified by Price *et al.* (1978), using the vanillin-hydrochloric acid reagent and catechin as the standard.

The AAs were determined by ion-exchange chromatography by employing a LKB 4151 Alpha Plus Amino acid Analyzer[3] equipped with an LKB 4029 Programmer and a 3393A Hewlett-Packard Integrator[4] following hydrolysis of the samples with 6 N HCl at 110 C for 24 hr (Andrews and Baldar, 1985). Methionine and cystine were determined using the performic oxidation method of Hirs (1967). Seed size was determined by weighing 100 seeds from each cultivar and was expressed as g/1000 seeds. All analyses were performed in duplicate.

***Digestibility Trial***

The precision-feeding technique with adult cockerels as described by Sibbald (1986) with modifications (Zhang *et al.*, 1994) was used to determine the uncorrected and nitrogen-corrected true metabolizable energy (TME and TMEn, respectively) and true amino acid availability (TAAA) values of peas.

Briefly, following a 28 hr fasting period, each pea sample was precision-fed (30 g/bird) to a group of 10 birds housed in individual metabolism cages (62.2 x 34.3 x 43.3 cm) in an environmentally controlled room. After 48 hr, all excreta from each bird was

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[3]LKB Biocgron Ltd., Cambridge Science Park, Cambridge, UK.

[4]Hewlett-Packard Co., Avondale, PA.

collected. The excreta samples were frozen, freeze-dried, ground to pass through a 1 mm sieve and analyzed for gross energy, nitrogen (Kjeldahl) and AA contents. An adiabatic oxygen bomb calorimeter[5] was used to measure gross energy of peas and excreta samples.

The TME and TMEn values were calculated according to the method of Sibbald (1986) and the TAAA were calculated as described by Sibbald (1979). Endogenous energy, nitrogen and amino acid values used in the calculations were obtained from pooled data for 30 unfed birds treated as described for the precision-fed birds. The TME, TMEn and TAAA values for each pea sample were determined in duplicate and each duplicate value represent a pooled determination from 10 birds.

### *Statistical Analysis*

The results obtained from the digestibility trial were subjected to analysis of variance using the General Linear Model (GLM) procedure of SAS® (SAS Institute, 1986). Duncan multiple range test (Duncan, 1955) was performed to compare treatment (cultivar) means. The ∝-level for significance was $P \leq 0.05$. Correlation coefficients were calculated to determine the relationship between protein content, seed weight, hull content and other chemical components of peas.

## RESULTS AND DISCUSSION

### *Composition of the Pea Cultivars*

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[5]Parr Instrument Co., Moline, IL.

The cultivars (Table 10) showed a very broad range of CP contents (207.5-264.0 g/kg). However, the mean protein content of 235.0±15.53 g/kg was similar to those reported in the literature (Marquardt and Bell, 1988; Savage and Deo, 1989). Although only 2 green-seeded and 2 brown-seeded cultivars were included in this study, it appeared that the variations in protein contents are not related to seed coat colour since the lowest and highest values are within the yellow-seeded cultivars. There was also no correlation ($r = -0.07$; $P \geq 0.05$) (Table 11) between protein content and seed size. This finding confirms the previous study by Ali-Khan and Youngs (1973) who obtained a correlation coefficient of -0.12 between protein and seed size and suggested that selection for high protein would not have any deleterious effects on seed size. This wide range of protein contents could be a reflection of the conditions under which the cultivars were grown or the inherent variental differences. In this regard, protein contents of peas are known to vary with soil type and nitrogen application (Igbasan *et al.* 1996b), location and year (Ali-Khan and Youngs, 1973) and genotypes (Matthews and Arthur, 1985).

The concentations of several AAs (expressed as g per 16 g N) varied among the cultivars (Table 12). There was no evidence to show that these variations are related to seed coat colour. Compared with cereal grains (NRC, 1994) and canola meal (Simbaya, 1995), on protein basis, these cultivars contained more lysine and less total sulphur amino acids (TSAA). The mean contents (g per 16 g N) of 7.5±0.20, 8.9±0.48 and 2.5±0.13 for lysine, arginine and TSAA respectively, were higher than the lysine (6.7 g) and arginine ( 7.8 g) contents but lower than the TSAA content (3.0 g) of soybean meal (Lalles, 1993). The concentration of AAs in the protein of peas is a function of the AA

TABLE 10. Chemical composition (g/kg DM) of the pea cultivars.

| Cultivars | CP | DF[1] | Starch | Ash | EE | Ca | P | Tannin[2] |
|---|---|---|---|---|---|---|---|---|
| Express | 207.5 | 210.8 | 436.6 | 24.2 | 17.9 | 0.6 | 2.9 | <1.0 |
| Highlight | 214.8 | 209.9 | 412.0 | 28.1 | 14.8 | 0.9 | 4.2 | <1.0 |
| Baroness | 225.7 | 203.2 | 433.9 | 32.2 | 18.2 | 0.9 | 5.3 | <1.0 |
| Titan | 225.9 | 200.2 | 436.8 | 29.6 | 18.5 | 0.6 | 5.1 | <1.0 |
| Fluo | 229.4 | 202.0 | 415.3 | 29.3 | 21.3 | 0.8 | 5.1 | <1.0 |
| Montana | 243.3 | 196.3 | 397.8 | 35.3 | 13.4 | 0.8 | 4.4 | <1.0 |
| Impala | 245.2 | 192.3 | 400.8 | 30.6 | 14.3 | 0.8 | 3.6 | <1.0 |
| Tara | 264.0 | 190.7 | 385.3 | 31.0 | 14.5 | 1.3 | 4.3 | <1.0 |
| Radley | 232.2 | 192.5 | 425.1 | 25.8 | 20.1 | 0.7 | 3.2 | <1.0 |
| Trump | 244.9 | 202.8 | 424.6 | 29.9 | 20.2 | 0.7 | 4.4 | <1.0 |
| Carman | 255.0 | 214.3 | 392.8 | 26.4 | 14.7 | 0.9 | 5.6 | 11.5 |
| Sirius | 231.9 | 223.1 | 415.3 | 29.5 | 12.4 | 0.8 | 3.0 | 41.0 |
| Mean ± sd | 235.0±15.53 | 203.2±9.44 | 414.7±16.77 | 29.3±2.85 | 16.7±2.88 | 0.8±0.18 | 4.3±0.88 | 5.2±11.17 |

CP = Crude protein; DF = Dietary fibre; EE = Ether extract; Ca = Calcium; P = Phosphorus.
[1] Includes non-starch polysaccharides, lignin and polyphenols, cell wall protein and cell wall ash.
[2] Tannin content was based on catechin equivalents and was detected only in pea hulls.

TABLE 11. Correlation coefficients (r) for the relationship between protein content and other chemical parameters of peas.

| | Protein, g/kg DM | |
|---|---|---|
| | r | Significance |
| Seed weight | -0.07 | ns[1] |
| Hull, %[3] | -0.07 | ns |
| Dietary fibre, g/kg[3] | -0.46 | ns |
| Starch | -0.78 | ** |
| Arginine, g per 16 g N | +0.79 | ** |
| Cystine, g per 16 g N | -0.60 | * |
| Histidine, g per 16 g N | -0.49 | ns |
| Isoleucine, g per 16 g N | -0.63 | * |
| Leucine, g per 16 g N | -0.59 | * |
| Lysine, g per 16 g N | -0.65 | * |
| Methionine, g per 16 g N | -0.33 | ns |
| Phenylalanine, g per 16 g N | -0.54 | ns |
| Threonine, g per 16 g N | -0.79 | ** |
| Valine, g per 16 g N | -0.52 | ns |

[1] Not significant.
[2] Correlation coefficient between hull and dietary fibre was + 0.70 ($P \leq 0.01$).
* $P \leq 0.05$; ** $P \leq 0.01$.

TABLE 12. Amino acid composition (g per 16 g N) of the pea cultivars.

| Cultivars | Ala | Arg | Asp | Glu | Gly | His | Ile | Leu | Lys | TSAA | Phe | Pro | Ser | Thr | Tyr | Val |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Express | 4.6 | 8.5 | 11.5 | 16.6 | 4.6 | 2.6 | 4.7 | 7.5 | 7.7 | 2.8 | 5.2 | 4.4 | 4.9 | 4.3 | 3.6 | 5.1 |
| Highlight | 4.5 | 8.5 | 11.4 | 16.5 | 4.7 | 2.6 | 4.4 | 7.2 | 7.6 | 2.6 | 4.9 | 4.5 | 5.0 | 4.1 | 3.6 | 4.9 |
| Baroness | 4.6 | 8.6 | 11.9 | 16.7 | 4.5 | 2.5 | 4.6 | 7.1 | 7.8 | 2.5 | 5.0 | 4.5 | 4.9 | 3.9 | 3.7 | 5.0 |
| Titan | 4.6 | 8.6 | 11.8 | 16.7 | 4.6 | 2.5 | 4.6 | 7.2 | 7.8 | 2.5 | 5.0 | 4.9 | 4.9 | 4.0 | 3.5 | 5.0 |
| Fluo | 4.4 | 8.7 | 11.7 | 16.4 | 4.3 | 2.5 | 4.5 | 7.0 | 7.3 | 2.5 | 4.8 | 4.5 | 4.8 | 3.8 | 3.5 | 5.2 |
| Montana | 4.3 | 8.7 | 11.7 | 17.3 | 4.3 | 2.7 | 4.4 | 7.2 | 7.4 | 2.4 | 4.8 | 4.3 | 4.8 | 3.8 | 3.5 | 5.0 |
| Impala | 4.2 | 9.3 | 11.5 | 16.8 | 4.3 | 2.4 | 4.5 | 7.3 | 7.2 | 2.7 | 4.9 | 4.3 | 4.8 | 3.7 | 3.4 | 4.8 |
| Tara | 4.3 | 9.7 | 12.0 | 16.7 | 4.3 | 2.3 | 4.3 | 6.9 | 7.3 | 2.3 | 4.9 | 4.3 | 4.8 | 3.8 | 3.4 | 4.7 |
| Radley | 4.4 | 8.6 | 11.8 | 16.3 | 4.4 | 2.8 | 4.6 | 7.2 | 7.3 | 2.5 | 4.8 | 4.4 | 4.8 | 3.9 | 3.7 | 4.7 |
| Trump | 4.2 | 9.2 | 11.5 | 16.7 | 4.2 | 2.3 | 4.6 | 7.2 | 7.4 | 2.4 | 4.9 | 4.3 | 4.7 | 3.6 | 3.5 | 4.9 |
| Carman | 4.2 | 9.9 | 11.5 | 16.7 | 4.2 | 2.5 | 4.4 | 7.0 | 7.4 | 2.4 | 4.8 | 4.3 | 5.0 | 3.8 | 3.2 | 4.9 |
| Sirius | 4.4 | 8.5 | 11.9 | 16.6 | 4.4 | 2.4 | 4.6 | 7.0 | 7.5 | 2.6 | 4.8 | 4.3 | 4.9 | 3.9 | 3.3 | 4.8 |
| Mean | 4.4 | 8.9 | 11.7 | 16.7 | 4.4 | 2.5 | 4.5 | 7.2 | 7.5 | 2.5 | 4.9 | 4.4 | 4.9 | 3.9 | 3.5 | 4.9 |
| Sd | 0.15 | 0.48 | 0.19 | 0.24 | 0.16 | 0.14 | 0.11 | 0.16 | 0.20 | 0.13 | 0.12 | 0.17 | 0.09 | 0.18 | 0.14 | 0.14 |

TSAA = Total sulphur amino acids (methionine + cystine).

composition of the storage proteins (Gueguen and Barbot, 1988). The main storage proteins in peas are albumins and globulins (Casey, 1983). The AA profiles of these protein fractions vary considerable among pea cultivars (Gueguen and Barbot, 1988), which may explain the variations in AA concentrations observed in the present study. High concentration of lysine and low concentration of TSAA in peas are often attributed to the AA composition of globulins, vicilin and legumin, which contribute above 80% of the total seed proteins in peas and are high in lysine but low in TSAA (Croy *et al.* 1980). In the context of meeting the dietary requirement of indispensable AAs for poultry (NRC, 1994), cereal grains, canola meal and peas are nutritionally complementary in that those AAs deficient in one (lysine in cereals and canola and sulphur AAs in peas) being adequate in the other.

The protein quality, in terms of indispensable AA concentrations in protein, decreased as protein levels increased in peas. As indicated in Table 11, the concentrations of most indispensable AAs were negatively correlated to seed protein content. Only arginine ($r = 0.79$) had positive correlation with protein content. The correlations were not significant ($P \geq 0.05$) for histidine, methionine, phenylalanine and valine. Similar relationships between AA concentrations and seed protein content have been reported by Holt and Sosulski (1979) and Igbasan *et al.* (1996). The concentration of arginine could also be explained from the AA composition of globulins (the main storage protein in peas) which contain more arginine and non-essential AAs than albumins, whose contents of other essential AAs are higher than globulins (Monti, 1983). An increase in the amount of globulins as the protein content increases would account for the arginine-protein

relationship observed here.

The pea cultivars were found to contain relatively high starch contents (385.3-436.8 g/kg) which are similar to those reported by Gatel and Grosjean (1990). Starch is the main dietary source of energy and these high levels therefore, indicate the importance of peas as an energy supplement in livestock and poultry feeding. The National Research Council (NRC, 1988) documented that digestible energy content of peas in pigs is higher than that of barley and comparable to that of wheat. However, in poultry, pea starch is less digestible than starch in any cereal grains (Longstaff and McNab, 1987) and hence of lower metabolizable energy value (Conan and Carre, 1989). A significant ($P \leq 0.01$) negative relationship (r = -0.78) between starch and protein contents was observed (Table 11). Most of the cultivars that were high in starch were relatively low in protein. However, an increase in the starch content of a particular cultivar cannot fully account for a similar decrease in its protein content. An observation which has been reported by Reichert and MacKenzie (1982).

The dietary fibre contents varied between 191 and 223 g/kg with a mean and standard deviation of 203±9.4 g/kg. The fibre values reported in the current study were slightly higher than the value (188 g/kg) reported by Wright *et al.* (1984). Such variation in results may in part, be attributed to the differences in analytical methods. The method used by these authors did not account for cell wall protein and ash and lignin with associated polyphenols. In the present study, values for dietary fibre content were slightly higher in the brown-seeded than in the yellow- or green-seeded pea cultivars.

The composition of the dietary fibre, as presented in Table 13, showed that NSP

constituted the major component (133-151 g/kg). Other components included cell wall protein (19.3-31.6 g/kg) and ash (2.6-6.4 g/kg) and lignin with associated polyphenols (25.6-44.5 g/kg). There were no major differences in NSP, cell wall protein and ash contents of yellow-, green- and brown-seeded cultivars. The only difference was the amounts of lignin with associated polyphenols which were higher in the brown-seeded cultivars. These differences may arise from the content of polyphenols (tannin) in the brown-seeded cultivars. This may also partly explain the relatively high content of dietary fibre in the brown-seeded cultivars. The regression of protein and dietary fibre contents showed a weak ($P \geq 0.05$) and negative correlation coefficient (r = -0.46), which suggests that the dilution effect of dietary fibre on protein content and overall nutritive value of peas may not be significant. There was a strong positive correlation (r = 0.70; $P \leq 0.01$) (see footnote in Table 11) between fibre and hull contents of peas which may also suggest the importance of dehulling to further improve the nutritive worth of peas.

The amounts of NSP (133-151 g/kg) were similar to those (100-150 g/kg) reported by Cerning-Beroard and Filiatre-Verel (1979) and Reichert (1981). As shown in Table 14 and judging from the contents of non-starch glucose, it is evident that cellulose is the predominant polysaccharide in peas. Uronic acid and arabinose residues are also present in appreciable quantities. Other components like xylose, galactose, mannose and rhamnose are present in minor quantities. These results were similar to those reported by Brillouet and Carre (1983) and Aman and Graham (1987). Our data (not presented here) and those of Reichert (1981) showed that most of the cellulose is found in the hulls. In the current study, the hull fraction contributed between 8.1 and 12% (Table 1) of the

TABLE 13. Composition of dietary fibre of the pea cultivars, g/kg DM.

| Cultivars | NSP | Cell wall protein | Cell wall ash | Lignin & polyphenols | Total |
|---|---|---|---|---|---|
| Express | 151.0 | 25.0 | 4.3 | 30.5 | 210.8 |
| Highlight | 145.0 | 26.5 | 4.7 | 33.7 | 209.9 |
| Baroness | 146.4 | 22.8 | 4.3 | 29.7 | 203.2 |
| Titan | 136.6 | 25.1 | 5.6 | 32.9 | 200.2 |
| Fluo | 136.0 | 24.3 | 5.6 | 36.1 | 202.0 |
| Montana | 140.2 | 25.1 | 4.0 | 27.0 | 196.3 |
| Impala | 137.7 | 23.0 | 2.8 | 28.8 | 192.3 |
| Tara | 133.4 | 25.1 | 4.8 | 27.4 | 190.7 |
| Radley | 145.0 | 19.3 | 2.6 | 25.6 | 192.5 |
| Trump | 141.5 | 23.0 | 3.6 | 34.7 | 202.8 |
| Carman | 137.4 | 31.6 | 3.9 | 41.4 | 214.3 |
| Sirius | 149.2 | 23.0 | 6.4 | 44.5 | 223.1 |
| Mean ± sd | 141.6±5.41 | 24.5±2.78 | 4.4±1.08 | 32.7±5.56 | 203.2±9.44 |

NSP = Non-starch polysaccharides.

TABLE 14. Non-starch polysaccharide profiles (% of Total) of the pea cultivars.

| Cultivars | Rhamnose | Arabinose | Xylose | Mannose | Galactose | Glucose | Uronic acids |
|---|---|---|---|---|---|---|---|
| Express | 0.4 | 18.4 | 5.5 | 0.5 | 3.4 | 48.5 | 23.3 |
| Highlight | 0.4 | 20.6 | 6.1 | 0.5 | 3.9 | 46.9 | 21.5 |
| Baroness | 0.6 | 17.8 | 5.2 | 0.4 | 4.7 | 48.2 | 23.0 |
| Titan | 0.5 | 18.5 | 6.9 | 0.5 | 3.4 | 47.1 | 23.1 |
| Fluo | 0.4 | 21.5 | 7.1 | 0.6 | 4.6 | 41.1 | 24.7 |
| Montana | 0.4 | 21.4 | 7.0 | 0.5 | 4.6 | 44.4 | 21.7 |
| Impala | 0.5 | 19.1 | 6.0 | 0.5 | 4.0 | 47.1 | 22.9 |
| Tara | 0.4 | 19.8 | 7.1 | 0.6 | 3.7 | 48.5 | 19.9 |
| Radley | 0.4 | 17.2 | 5.8 | 0.4 | 3.8 | 51.0 | 21.4 |
| Trump | 0.5 | 18.8 | 6.9 | 0.5 | 3.7 | 49.5 | 20.2 |
| Carman | 0.4 | 19.0 | 6.8 | 0.5 | 4.5 | 44.7 | 24.0 |
| Sirius | 0.4 | 18.3 | 5.5 | 0.6 | 4.3 | 51.7 | 19.2 |
| Mean ± sd | 0.4±0.06 | 19.2±1.31 | 6.3±0.68 | 0.5±0.06 | 4.1±0.45 | 47.4±2.82 | 22.1±1.64 |

dry matter in peas. The relatively high concentrations of uronic acid and arabinose residues, probably derived from cotyledons as noted by Brillouet and Carre (1983), indicate that pectic-type substances are important components of the cell walls of peas.

The two brown-seeded cultivars, Carman and Sirius, contained appreciable quantities of tannins while the yellow- and green-seeded cultivars were devoid of tannins. The tannins were located in the seed coat. Griffiths (1981) has reported the presence of tannins in dark-flowered pea varieties. The author also showed that tannin content of peas is quite variable and the dark-flowered varieties can contain up to 10 times more than the white-flowered varieties.

The cultivars were almost devoid of fat (ether extract) and calcium but well provided with phosphorus. If available, peas can serve as a good source of phosphorus for monogastric animals. However, like other legumes, peas contain phytic acid which affects bioavailability of phosphorus (Manan *et al.* 1987). The fat, calcium and phosphorus contents were similar to those reported by Savage and Deo (1989).

***Digestibility Trial***

The TME and $TME_n$ values of the cultivars of peas obtained with adult cockerels are given in Table 15. The cultivars showed significant ($P \leq 0.05$) differences in TME and $TME_n$ values. The TME values ranged from 11.6 to 13.3 MJ/kg with a mean and standard deviation of 12.7±0.54 while the $TME_n$ values ranged from 11.0 to 12.9 MJ/kg with a mean and standard deviation of 12.2±0.54. Nitrogen correction was recommended by Wolynetz and Sibbald (1984) to allow adjustment to be made for differences between

TABLE 15. Uncorrected and nitrogen-corrected true metabolizable energy (TME) values of the pea cultivars.

| Cultivars | TME, MJ/kg | $TME_n$, MJ/kg |
|---|---|---|
| Express | 12.8bc | 12.3bc |
| Highlight | 13.0abc | 12.5b |
| Baroness | 13.3a | 12.9a |
| Titan | 13.1ab | 12.6b |
| Fluo | 12.9bc | 12.4bc |
| Montana | 13.0abc | 12.5b |
| Impala | 12.2d | 11.7d |
| Tara | 12.4d | 11.8d |
| Radley | 13.3a | 12.6b |
| Trump | 11.8e | 11.4e |
| Carman | 12.8bc | 12.2c |
| Sirius | 11.6e | 11.0f |
| Mean ± sd | 12.7±0.54 | 12.2±0.54 |

a-f Means within the same column followed by the same letters are not significantly different ($P \geq 0.05$)

the test fed and the starved birds. Because nitrogen retention was negative the $TME_n$ values of peas were lower than the TME values. Nitrogen correction resulted in a 3.4-5.3% reduction in the TME values of peas. The lowest metabolizable energy value was obtained from the cultivar (Sirius) that contained some tannins. However, tannins may not be the only factor responsible for the low energy value because Trump, another cultivar which contained no tannin, was equally low in metabolizable energy value. Lacassagne *et al.* (1988) and Jansman (1993) have shown that the effect of tannins on energy digestion is less important.

The data provided by Savage and Deo (1989) gave a range of 12.31 to 15.63 MJ/kg values for TME and 10.09 to 12.83 MJ/kg values for $TME_n$ of raw peas. Also, Askbrant (1988) evaluated some samples of white-flowered peas and reported a range of 12.48 to 15.60 MJ/kg values for TME. The TME and $TME_n$ values obtained in the present study are in agreement with those given by these authors. The metabolizable energy values of peas reported here and by other authors showed considerable variability, which may arise from the use of different cultivars of peas, variable starch contents and starch digestibility and variable concentrations of antinutritional factors in peas used.

Compared with cereal grains (NRC, 1994), canola meal (Askbrant, 1988; Simbaya, 1995) and soybean meal (Askbrant, 1988; NRC, 1994), the mean $TME_n$ value ($12.2 \pm 0.54$ MJ/kg) of these cultivars is comparable to that of barley, higher than that of canola meal and soybean meal but lower than that of wheat. Based on the energy values obtained in the present study, peas can provide a significant portion of the energy requirement of poultry.

The results of AA bioavailabilities shown in Table 16 indicated major differences among the cultivars ($P \leq 0.05$). The mean availabilities of different AAs ranged from 89.6 to 75.9% with TSAA having the lowest value and glutamic acid having the highest value. A closer look at individual AAs revealed a trend toward lower AA bioavailability values in the brown-seeded cultivars (cv.Carman and Sirius). This may in part, be attributed to the presence of tannins in these cultivars. There is overwhelming evidence in the literature to demonstrate that tannins reduce AA digestibilities (Marquardt, 1989; Jansman, 1993). The mean availability value for individual AAs was lower than the tabulated value (NRC, 1994) for soybean meal (dehulled and processed) but comparable to that of raw soybeans (Heartland lysine, 1995) and canola seed meals (Simbaya, 1995).

The present study shows that the chemical composition of the pea cultivars varied widely. Starch and dietary fibre were negatively correlated with protein content and they accounted for most of the difference in protein content. The protein quality, in terms of the concentrations of indispensable AAs, decreased as protein levels increased. Only the brown-seeded cultivars contained tannins and these seemed to have potential effects on the nutritive quality of these cultivars. The cultivars were relatively high in metabolizable energy contents which make them potential energy supplements for poultry diets. The bioavailabilities of individual AAs varied considerably among the cultivars and they were always lower with brown-seeded cultivars.

TABLE 16. True amino acid availabilities (%) of the pea cultivars[1].

| Cultivars | Ala | Arg | Asp | Glu | His | Ile | Leu | Lys | TSAA | Phe | Pro | Ser | Thr | Tyr | Val |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| YELLOW PEAS | | | | | | | | | | | | | | | |
| Express | 85b | 89ab | 89bc | 92a | 88bc | 80g | 89ab | 83b | 76cd | 85a | 85ab | 88a | 83bc | 86.0a | 82ed |
| Highlight | 84c | 89bc | 89c | 91ab | 89ab | 82f | 88abc | 81c | 78c | 85a | 86a | 88a | 87a | 87.0a | 81e |
| Baroness | 84c | 91a | 91ab | 90ab | 88bc | 87bc | 88abc | 83b | 77cd | 82bc | 84c | 87a | 84b | 84.4a | 86ab |
| Titan | 86b | 91a | 91a | 91ab | 90a | 90a | 90a | 83b | 86a | 86a | 84bc | 88a | 85b | 84.5a | 87a |
| Fluo | 83c | 88cde | 88c | 90ab | 84def | 85de | 88abc | 79d | 77cd | 81bc | 84bc | 85b | 82cd | 82.8a | 85abc |
| Montana | 88a | 83f | 90bc | 90b | 85de | 89b | 88bc | 82bc | 82b | 86a | 85bc | 83c | 83cd | 83.8a | 81e |
| Impala | 82d | 87cde | 91ab | 90b | 89ab | 85de | 87c | 86a | 80b | 82b | 78e | 85b | 80e | 83.5a | 82ed |
| Tara | 84c | 86e | 91a | 91ab | 87c | 86cd | 88abc | 78de | 78c | 80c | 83c | 86ab | 85b | 84.0a | 84bc |
| GREEN PEAS | | | | | | | | | | | | | | | |
| Radley | 84c | 88bc | 89c | 90ab | 82f | 84e | 88bc | 77ef | 75d | 82b | 78e | 87ab | 82de | 84.3a | 83cde |
| Trump | 84c | 87ed | 88c | 90ab | 90a | 84e | 87bc | 76f | 76cd | 83b | 80d | 87ab | 80e | 86.5a | 82e |
| BROWN PEAS | | | | | | | | | | | | | | | |
| Carman | 81d | 84f | 86d | 86c | 84ef | 81fg | 85d | 70h | 64e | 82b | 79e | 82c | 75f | 78.6a | 82de |
| Sirius | 74e | 84f | 83e | 84d | 86d | 76h | 79e | 73g | 64e | 72d | 69f | 75d | 70g | 72.2b | 71f |
| Mean | 83 | 87 | 89 | 90 | 879 | 84 | 87 | 79 | 76 | 82 | 81 | 85 | 81 | 83.1 | 82 |
| Sd | 3.2 | 2.4 | 2.4 | 2.1 | 2.6 | 3.8 | 2.7 | 4.3 | 6.1 | 3.6 | 4.6 | 3.7 | 4.4 | 3.88 | 3.8 |

[1] Corrected for endogenous amino acid losses.
TSAA = Total sulphur amino acids (methionine + cystine).
a-h Means within the same column followed by the same letters are not significantly ($P \geq 0.05$) different.

**MANUSCRIPT 2**

# PROTEIN QUALITY OF PEAS AS INFLUENCED BY LOCATION, NITROGEN APPLICATION AND SEED INOCULATION

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**ABSTRACT** A study was conducted to evaluate the contribution of location, N application and *Rhizobium* seed inoculation to variations in seed protein content and amino acid (AA) compositon of field peas. The magnitude of AA variations with protein level and the nature of the relationships that are involved were determined. Regression equations to predict AAs from protein were developed for the cultivar Bohatyr. The experiments were carried out at two locations in southern Manitoba in 1994. The levels of N fertilization investigated were: 56, 75, 100, 125, 150, 200, 250 and 300 kg/ha. At each level of N application, seeds planted were either *Rhizobium* inoculated or not inoculated. The combination of location, fertilizer treatments and inoculation yielded 192 samples for chemical analyses. The samples were analyzed for dry matter (DM), N and AA contents. Location and N fertilization had significant ($P \leq 0.001$) effects on seed protein content and AA composition. Seed protein content increased with increasing levels of N application. The response of protein to fertilization was not the same in both locations as evidenced from the presence of interaction ($P \leq 0.01$) between location and N application. Except for methionine and cystine, percent AAs in DM increased with increasing levels of N application. The effects of N application on the concentrations of methionine and cystine were not consistent. On a protein basis, the concentrations of AAs decreased with increasing levels of N application. The only exception was arginine which increased in concentration. There was no effect ($P \geq 0.05$) of seed inoculation observed in this study. Strong positive correlations ($r > 0.80$) between seed protein content and AA concentrations expressed as percent of DM were found for all AAs except for methionine ($r = 0.76$) and cystine ($r = 0.51$). When AA concentrations are expressed as g per 16 g

N, 15 of the 17 AAs were negatively correlated to seed protein content. Only arginine (r = 0.78) and aspartic acid (r = 0.17) had positive correlations. The regression equations developed from this study could be used to predict the concentrations of AAs except methionine and cystine for the cultivar Bohatyr once the protein content is known.

**Key words:** Field peas, location, N-fertilization, inoculation, protein, amino acids.

## INTRODUCTION

The growing feed pea (*Pisum sativum* L.) market in Western Europe, the main export market for Canadian grown peas and the increase in domestic consumption of peas and pea products have encouraged farmers to increase the area sown to field peas in Western Canada. In 1984 an estimated 73,600 ha of land was sown to field peas whereas in 1994 over 640,000 ha of land was devoted to pea production (Slinkard, 1994) representing an increase of 770% in one decade.

Despite this dramatic increase in production, peas remain an under-exploited feedstuff in animal nutrition. Livestock and poultry diets in Western Canada are still based on wheat, barley or corn with imported soybean meal as the major protein supplement. Many nutritionists and feed manufacturers are not willing to incorporate peas into regular dietary formulation because they are concerned about the great variation in the nutritive quality of peas.

Several authors (Sosulski, 1971; Ali-Khan and Youngs, 1973; Sosulski *et al.*,

1974; Cousin, 1983; Holt and Sosulski, 1979; Monti, 1983; Muller, 1983) have noted differences in protein content and amino acid (AA) composition within and among pea cultivars. More recently, Igbasan *et al.* (1994) evaluated twelve newly registered pea cultivars in Western Canada for nutritional quality and observed that there were considerable variations in protein content and AA composition among these cultivars. Due to variation in AA composition, protein quality becomes very important when peas are grown specifically for their protein for human or animal consumption because the biological value of any protein is dependent on the quantity and availability of essential amino acids.

Factors including location (Sosulski, 1971; Ali-Khan and Youngs, 1973; Matthews and Arthur, 1985), level of nitrogen (N) application (Eppendorfer and Bille, 1974; Trevino and Murray, 1975; Cowan, 1979; Andersen *et al.,* 1983), variety and year of harvesting (Ali-Khan and Youngs, 1973; Holt and Sosulski, 1979) and genotype (Muller, 1983; Casey, 1983; Matthews and Arthur, 1985; Kalloo, 1993) have been found to influence protein content and AA composition of peas. While a number of these workers agreed that the environmental components (location and N application) played a dominant role in the variability of protein content of peas, others reported that environment had only a minimal effect on pea protein content with genotype having a greater influence.

The present study was undertaken to further evaluate the contribution of location, N fertilization and *Rhizobium* seed inoculation to variation in protein and AA composition of field peas. The magnitude of AA variations with protein level and the nature of the relationships that are involved were determined. The regression equations to predict AAs

from protein were also developed for the cultivar Bohatyr, since these equations have been reported to be variety specific (Holt and Sosulski, 1979).

## MATERIALS AND METHODS

The experiments were carried out at two locations, near Morden, Manitoba in 1994. Location 1 was loam soil, with a pH of 7.4 and electrical conductivity of 0.3 $mSm^{-1}$ while location 2 was a sandy loam with a pH of 8.3 and electrical conductivity of 0.4 $mSm^{-1}$. The cultivar Bohatyr, developed in the Czech republic and recently registered in Western Canada, was used in this study.

Increasing levels of N fertility were established by supplementing available soil N (56 kg/ha at both locations, based on soil analysis) with urea fertilizer (46-0-0) to total available N levels of 56, 75, 100, 125, 150, 200, 250 and 300 kg/ha. At each level of N application, seeds planted were either *Rhizobium* inoculated or not inoculated. The inoculation was carried out with an effective strain of commercial seed inoculant[1]. Seeds were sown on May 27, 1994 at the rate of 150 kg/ha.

The experimental design employed in this study was a split-plot with six replications. Each plot consisted of four rows, 0.3 m apart, 5 m long and 1.2 m between plots. The combination of 2 locations, 8 fertilizer treatments and 2 inoculation (with or without) treatments with 6 replications per treatment yielded 192 samples for chemical analyses.

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[1]N-prone®, Philombios, Saskatoon, Saskatchewan.

The samples were analysed for dry matter (DM), N and AA contents. The DM content was determined according to the procedure of the Association of Official Analytical Chemists (AOAC, 1984). The N content was determined using the Kjeldahl procedure (AOAC, 1984). Crude protein content was estimated by multiplying N content (%) by a 6.25 conversion factor.

The AA composition was determined as outlined by Andrews and Baldar (1985) with performic acid oxidation of cystine and methionine according to Hirs (1967). Final analysis was carried out by employing an LKB 4151 Alpha Plus Amino acid Analyzer[2] equiped with an LKB 4029 Programmer and a 3393A Hewlett-Packard Integrator[3]. Values are reported here both as percent AA in DM and as g AA per 16 g N. All chemical analyses were performed in duplicate.

Data were subjected to analysis of variance using the General Linear Models (GLM) procedure of the Statistical Analysis System, Institute, Inc. Programme (SAS® Institute, Inc. 1986). To assess the relationship between AA content and seed protein, regression and correlation statistics were performed on the data obtained from one experimental location using the procedures of SAS® (1986).

## RESULTS AND DISCUSSION

### *Seed Protein Content*

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[2]LKB Biochron Ltd., Cambridge Science Park, Cambridge, UK.

[3]Hewlett-Packard Co., Avondale, PA.

The seed protein content as influenced by location, N application and seed inoculation are presented in Tables 17, 18 and Figure 1. Seed protein contents varied from 24.8 to 26.6 percent and from 22.3 to 25.8 percent for location 1 and location 2, respectively. There was a consistent and gradual increase in percent protein with increasing levels of N fertilization at both locations. Except for N application at 300 kg/ha, seed inoculation with N-fixing bacteria increased protein content slightly above non-inoculated seeds at the same fertilizer level ($P \geq 0.05$).

Table 19 shows the degrees of significance of effects of location, N application and seed inoculation and their interactions on seed protein content. Location and N application affected seed protein significantly ($P \leq 0.001$). There was no significant ($P \geq 0.05$) effect of seed inoculation observed in this study.

Protein content increased with each increment of N fertilizer (figure 1), which suggests that the protein content of field peas is a function of the N status of the soil. The response of protein to fertilization was not the same in both locations and the presence of interaction (Table 19) between location and fertilizer further indicates that N application produced different responses in the two locations. Since the initial soil N content was the same, it is probable that the difference in response is as a result of other soil specific factors. Two specific differences between the soils may have contributed to the differences in protein response, location 2 had a higher pH and was more prone to drought than location 1.

These results are consistent with findings reported by Sosulski *et al.* (1974), McLean *et al.* (1974), Eppendorfer and Bille (1974), Trevino and Murray (1975) and

TABLE 17. Seed protein and amino acid concentrations of field peas as influenced by Nitrogen application, Location 1.

| | | Nitrogen Application, Kg/ha | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 56 | 75 | 100 | 125 | 150 | 200 | 250 | 300 |
| Seed Protein, % | (-)[a] | 24.75 | 24.81 | 25.13 | 25.31 | 25.63 | 25.88 | 25.94 | 26.56 |
| | (+)[b] | 24.81 | 25.06 | 25.31 | 25.69 | 25.50 | 26.19 | 26.44 | 26.50 |
| | | Amino acid concentration, g per 16 g N | | | | | | | |
| Alanine | | 4.33 | 4.33 | 4.35 | 4.29 | 4.23 | 4.29 | 4.29 | 4.22 |
| Arginine | | 8.98 | 9.38 | 9.83 | 9.73 | 9.61 | 9.85 | 10.01 | 10.04 |
| Aspatic acid | | 10.75 | 10.75 | 10.59 | 10.75 | 10.72 | 10.56 | 10.96 | 10.78 |
| Cystine | | 1.45 | 1.44 | 1.47 | 1.52 | 1.56 | 1.44 | 1.45 | 1.49 |
| Glutamic acid | | 17.22 | 16.93 | 16.75 | 16.97 | 17.15 | 16.85 | 16.78 | 16.99 |
| Glycine | | 4.33 | 4.29 | 4.31 | 4.29 | 4.25 | 4.28 | 4.33 | 4.35 |
| Histidine | | 2.43 | 2.43 | 2.44 | 2.46 | 2.47 | 2.43 | 2.44 | 2.47 |
| Isoleucine | | 4.58 | 4.46 | 4.38 | 4.43 | 4.43 | 4.48 | 4.46 | 4.38 |
| Leucine | | 7.34 | 7.34 | 7.20 | 7.18 | 7.15 | 7.16 | 7.14 | 7.09 |
| Lysine | | 7.48 | 7.45 | 7.41 | 7.43 | 7.43 | 7.41 | 7.41 | 7.39 |
| Methionine | | 1.01 | 1.02 | 1.02 | 1.04 | 1.06 | 1.01 | 0.98 | 0.99 |
| Phenylalanine | | 4.98 | 4.89 | 4.84 | 4.90 | 4.93 | 4.86 | 4.81 | 4.84 |
| Proline | | 4.36 | 4.28 | 4.23 | 4.33 | 4.42 | 4.39 | 4.31 | 4.26 |
| Serine | | 4.89 | 4.98 | 5.05 | 4.99 | 4.89 | 4.88 | 4.91 | 4.92 |
| Threonine | | 3.80 | 3.81 | 3.79 | 3.76 | 3.75 | 3.83 | 3.85 | 3.77 |
| Tyrosine | | 3.55 | 3.37 | 3.35 | 3.40 | 3.42 | 3.45 | 3.46 | 3.46 |
| Valine | | 5.13 | 5.11 | 5.04 | 5.03 | 5.01 | 4.97 | 4.95 | 4.96 |

[a] Without inoculation.
[b] With inoculation.

TABLE 18. Seed protein and amino acid concentrations of field peas as influenced by nitrogen application, Location 2.

| | | Nitrogen Application, Kg/ha | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 56 | 75 | 100 | 125 | 150 | 200 | 250 | 300 |
| Seed Protein, % | (-)[a] | 22.25 | 22.31 | 23.06 | 23.31 | 23.75 | 24.00 | 24.88 | 25.75 |
| | (+)[b] | 22.44 | 22.88 | 23.13 | 23.69 | 23.94 | 24.44 | 25.00 | 25.63 |
| | | Amino acid concentration, g per 16 g N | | | | | | | |
| Alanine | | 4.57 | 4.46 | 4.37 | 4.36 | 4.33 | 4.35 | 4.29 | 4.27 |
| Arginine | | 8.41 | 8.51 | 8.63 | 8.94 | 9.63 | 9.92 | 9.65 | 9.41 |
| Aspartic acid | | 10.69 | 10.49 | 10.39 | 11.06 | 10.78 | 11.11 | 10.71 | 10.59 |
| Cystine | | 1.60 | 1.61 | 1.64 | 1.57 | 1.51 | 1.48 | 1.44 | 1.57 |
| Glutamic acid | | 16.81 | 16.86 | 16.90 | 16.71 | 16.93 | 17.04 | 16.55 | 16.82 |
| Glycine | | 4.47 | 4.48 | 4.49 | 4.38 | 4.36 | 4.34 | 4.24 | 4.22 |
| Histidine | | 2.48 | 2.50 | 2.51 | 2.40 | 2.46 | 2.43 | 2.38 | 2.46 |
| Isoleucine | | 4.52 | 4.48 | 4.44 | 4.43 | 4.38 | 4.49 | 4.32 | 4.35 |
| Leucine | | 7.05 | 7.06 | 6.96 | 6.95 | 7.09 | 7.21 | 6.99 | 7.07 |
| Lysine | | 7.61 | 7.60 | 7.59 | 7.36 | 7.42 | 7.49 | 7.63 | 7.42 |
| Methionine | | 1.06 | 1.04 | 1.04 | 0.99 | 1.03 | 0.99 | 1.02 | 1.04 |
| Phenylalanine | | 4.96 | 4.92 | 4.91 | 4.81 | 4.86 | 4.90 | 4.74 | 4.71 |
| Proline | | 4.43 | 4.28 | 4.16 | 4.31 | 4.26 | 4.23 | 4.19 | 4.17 |
| Serine | | 4.97 | 4.89 | 4.91 | 4.74 | 4.76 | 4.83 | 4.99 | 4.92 |
| Threonine | | 3.98 | 3.94 | 3.93 | 3.90 | 3.80 | 3.76 | 3.83 | 3.79 |
| Tyrosine | | 3.58 | 3.49 | 3.41 | 3.46 | 3.46 | 3.51 | 3.27 | 3.31 |
| Valine | | 4.96 | 4.99 | 5.04 | 5.11 | 4.84 | 4.79 | 4.91 | 4.88 |

[a] Same as Table 17.
[b] Same as Table 17.

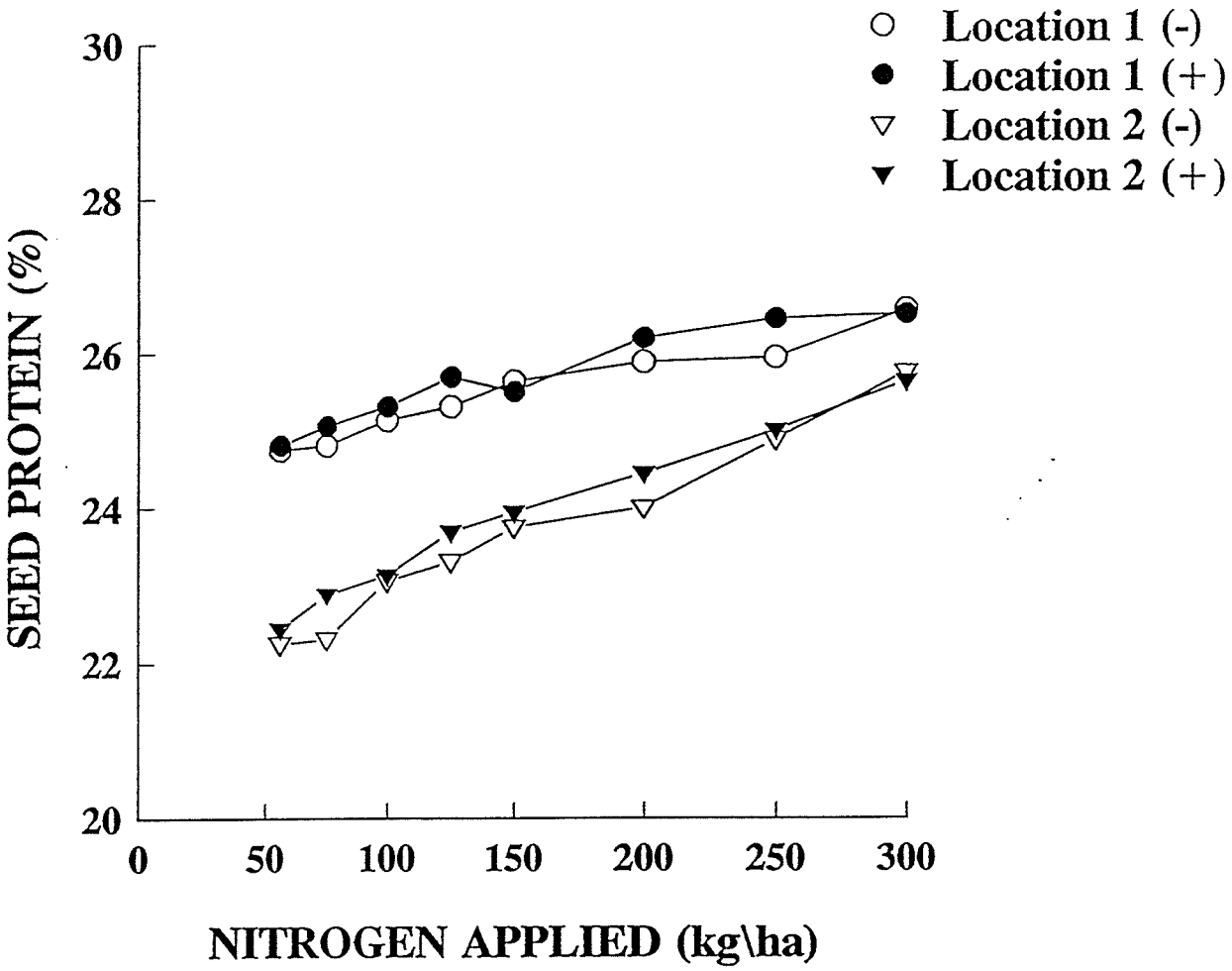


**Fig1. Effect of location,nitrogen application, with (+) and without (-) inoculation on seed protein content of field peas.**

TABLE 19. Degrees of significance (*F* test) of effects and interactions of N application (N), inoculation (I) and location (L) on seed protein and concentrations of amino acids of field peas[a].

| | N | L | I | N*L | N*I | L*I | N*L*I | N | L | I | N*L | N*I | L*I | N*L*I |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Seed protein | *** | *** | NS | ** | NS | NS | NS | - | - | - | - | - | - | - |
| | Amino acid, % of DM | | | | | | | Amino acid, g per 16 g N | | | | | | |
| Ala. | *** | *** | NS[b] | ** | NS | NS | NS | *** | *** | NS | *** | NS | NS | * |
| Arg. | *** | *** | ** | *** | *** | NS | ** | *** | *** | * | *** | *** | ** | *** |
| Asp. | *** | *** | ** | NS | NS | NS | NS | *** | * | NS | ** | ** | * | ** |
| Cys. | *** | *** | * | *** | ** | NS | *** | *** | ** | NS | *** | ** | * | *** |
| Glu. | *** | *** | NS | *** | NS | NS | NS | *** | *** | * | *** | * | ** | *** |
| Gly. | *** | *** | ** | NS | NS | NS | NS | *** | *** | * | *** | ** | ** | *** |
| His. | *** | *** | ** | ** | ** | NS | NS | *** | ** | *** | *** | ** | NS | *** |
| Ile. | *** | *** | NS | ** | NS | NS | NS | *** | *** | * | *** | *** | NS | *** |
| Leu. | *** | *** | NS | *** | NS | NS | NS | *** | *** | ** | *** | *** | NS | *** |
| Lys. | *** | *** | * | *** | NS | NS | NS | *** | *** | NS | *** | NS | NS | ** |
| Met. | *** | *** | NS | *** | ** | NS | ** | *** | *** | * | *** | ** | ** | *** |
| Phe. | *** | *** | NS | NS | NS | NS | NS | *** | *** | NS | *** | *** | ** | *** |
| Pro. | *** | *** | NS | * | * | NS | NS | *** | *** | ** | *** | *** | *** | *** |
| Ser. | *** | *** | NS | *** | NS | NS | NS | *** | *** | NS | *** | NS | NS | * |
| Thr. | *** | *** | NS | * | NS | NS | NS | *** | *** | NS | *** | * | NS | ** |
| Tyr. | *** | *** | NS | * | NS | NS | NS | *** | * | ** | *** | ** | ** | *** |
| Val. | *** | *** | NS | *** | NS | NS | NS | *** | *** | ** | *** | ** | NS | *** |

[a] Data from both experiments were combined.

[b] Not significant.

* Effect significant at $P = 0.05$.

** Effect significant at $P = 0.01$.

*** Effect significant at $P = 0.001$.

Andersen *et al.* (1983) who demonstrated that N fertilizers caused increases in pea protein content. Also Ali-Khan and Youngs (1973) and Matthews and Arthur (1985) revealed the apparent existence of considerable variation in pea protein contents as a result of environment and showed that varieties differed markedly in their response to environment.

Lack of significant response of protein to seed inoculation is a reflection of the effects of N fertilization on N-fixation. High levels of soil and fertilizer N can reduce or even inhibit nodulation and symbiotic N-fixation in legumes (Andersen *et al.*, 1983). McLean *et al.* (1974) and Cowan (1979) also reported that seed inoculation had no effect on seed protein content. Total N content of peas is about the same irrespective of the form of nitrogen available to the plants (Cowan, 1979).

***Seed Amino Acid Composition***

Data for seed AA composition are presented in Tables 17, 18 and figure 2. Except for methionine and cystine, percent of other AAs in DM increased (figure 2) with increasing levels of N application. The effects of N application on the concentrations of methionine and cystine were inconsistent in this study. From data in Tables 17 and 18, it is apparent that although the percentage of other AAs in DM may increase with N application, these AAs actually decreased in concentration on a protein basis. The only exception is arginine which strongly increased in concentration with increasing levels of N fertilization.

Table 19 shows the degrees of significance of effects and interactions of location, N application and seed inoculation on the concentrations of individual AAs. When

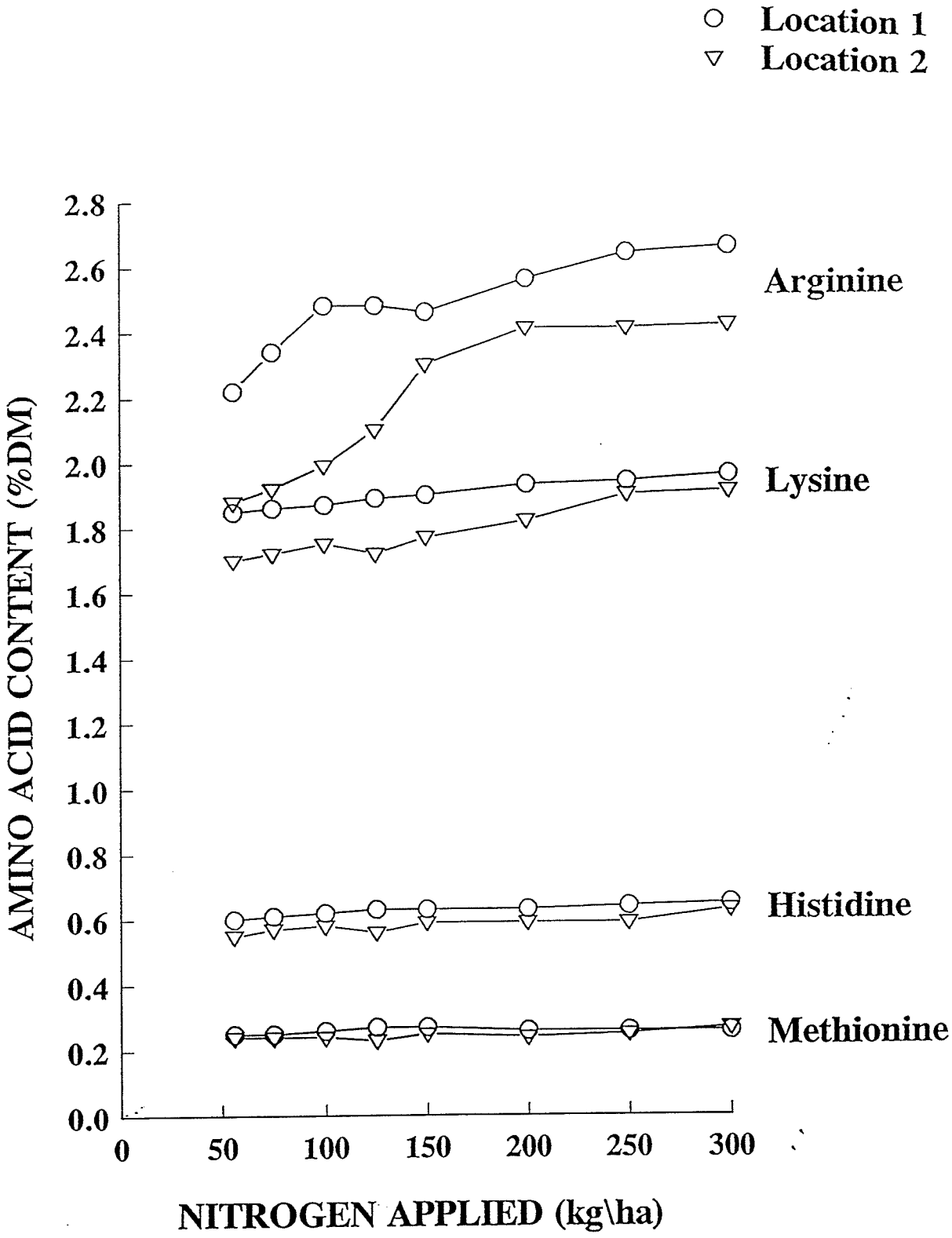


**Fig2. Interaction between nitrogen level and location on basic amino acid content of field peas.**

expressed as percent of DM and as g per 16 g N, location and N application strongly ($P \leq 0.001$) influenced the concentrations of all AAs. There were few significant differences in the levels of protein AAs as a result of seed inoculation. Significant interactions between location and fertilizer level were noted for most AAs, indicating that the fertilizer effect was not the same in both locations. Also, there were significant interactions between fertilizer level and inoculation for a few AAs. Three-way interactions between location, fertilizer level and inoculation were found to be significant for all AAs when expressed as g per 16 g N but the contributions of the sums of squares of these interactions to the total sums of squares were small and negligible.

Generally, AA composition changes according to protein level. Since seed protein content increases with increasing level of N application, it is expected that AA concentrations would also change. It is interesting to note that 9 of the 10 essential AAs (including cystine) decreased as a result of N fertilization. An increase in level of N fertilization is accompanied by an increase in the concentration of arginine. The decrease of essential AAs with N application has been reported for peas (Eppendorfer and Bille, 1974), faba beans (Eppendorfer, 1971) and barley (Pomeranz *et al.*, 1977).

The decrease in concentrations of essential AAs could be explained by the difference in deposition and composition of storage proteins in pea seeds. There are two types of storage proteins in peas, albumins and globulins in the ratio of 1:1.4 (Muller, 1983; Casey, 1983). Storage globulins contribute up to 80% of the seed proteins in peas and are principally consisting of vicilin and legumin (Boulter *et al.*, 1973). Except for arginine, albumin is higher in all essential AAs than globulin while globulin contains

more arginine and non-essential AAs than albumin (Bajaj *et al.*, 1971; Monti, 1983). Bajaj *et al.*, (1971) demonstrated that the greater proportion of the variation in biological value of pea protein could be attributed to differences in albumin content. It seems that the arginine content of globulin is influenced by environment and N application while the non-essential amino acid components (which did not change much in this study) and the composition of albumin are conditioned by genotype more than environment and N application. This observation has been reported by Kalloo (1993).

***Relationship Between Seed Amino Acid Composition and Seed Protein Content***

Table 20 shows regression equations, correlation coefficients (r) and degrees of significance of the relationship between concentrations of AAs and seed protein content. Data from location 2 were used in calculating these regression equations and correlation coefficients because it displayed wider variation in seed protein content which gives more reliable correlations. Strong positive correlations ($r > 0.80$) between seed protein content and AA concentrations expressed as percent of DM were found for all AAs except methionine ($r = 0.76$) and cystine ($r = 0.51$). When AA concentrations were expressed as g per 16 g N, 15 of the 17 AAs were negatively correlated to seed protein content. Only arginine ($r = 0.78$) and aspartic acid ($r = 0.17$) had positive correlations with protein content. The correlations were significant ($P \leq 0.05$) for alanine, arginine, cystine, glycine, histidine, isoleucine, phenylalanine, proline, threonine, tyrosine and valine but were not significant ($P \geq 0.05$) for aspartic acid, glutamic acid, leucine, lysine, methionine and serine.

TABLE 20. Regression equation and correlation coefficient (r) for the relationship between concentration of amino acid (y) and protein content (x) of seed of field peas[a].

| Amino acid | y = % amino acid in dry matter | | | y = g amino acid per 16 g N | | |
|---|---|---|---|---|---|---|
| | Regression equation | r | Significance | Regression equation | r | Significance |
| Ala. | y = 0.39+0.027x | 0.94 | *** | y = 6.15-0.075x | -0.87 | *** |
| Arg. | y = -2.28+0.187x | 0.93 | *** | y = -0.39+0.401x | 0.78 | ** |
| Asp. | y =- 0.19+0.115x | 0.91 | *** | y = 9.84+0.037x | 0.17 | NS[b] |
| Cys. | y = 0.21+0.006x | 0.51 | * | y = 2.41-0.036x | -0.60 | * |
| Glu. | y = 0.11+0.163x | 0.98 | *** | y = 17.52-0.029x | -0.22 | NS |
| Gly. | y = 0.47+0.024x | 0.96 | *** | y = 4.36-0.0123x | -0.96 | *** |
| His. | y = 0.13+0.019x | 0.90 | *** | y = 2.96-0.022x | -0.54 | * |
| Ile. | y = 0.27+0.033x | 0.97 | *** | y = 5.60-0.049x | -0.80 | *** |
| Leu. | y = 0.085+0.074x | 0.97 | *** | y = 7.10-0.013x | -0.18 | NS |
| Lys. | y = 0.16+0.068x | 0.95 | *** | y = 8.32-0.034x | -0.37 | NS |
| Met. | y = 0.057+0.008x | 0.76 | ** | y = 1.18-0.007x | -0.31 | NS |
| Phe. | y =0.40+0.032x | 0.95 | *** | y = 6.51-0.069x | -0.89 | *** |
| Pro. | y = 0.29+0.030x | 0.92 | *** | y = 5.49-0.052x | -0.67 | * |
| Ser. | y = 0.013+0.049x | 0.94 | *** | y = 5.04-0.006x | -0.07 | NS |
| Thr. | y = 0.33+0.025x | 0.93 | *** | y = 5.27-0.059x | -0.83 | *** |
| Tyr. | y = 0.10+0.038x | 0.81 | *** | y = 5.14-0.072x | -0.80 | *** |
| Val. | y = 0.25+0.039x | 0.90 | *** | y = 6.04-0.046x | -0.50 | * |

[a] Amino acid equations were based on location 2 experimental data.

[b] Not significant.

* Value of r significant at $P = 0.05$.

** Value of r significant at $P = 0.01$.

*** Value of r significant at $P = 0.001$.

Eppendorfer and Bille (1974) and Mosse *et al.* (1987) found strong positive correlations between AA concentrations (as percent of DM) and protein content for all AAs which agree well with the present results. Strong positive correlations were reported for arginine concentration by the same authors and Holt and Sosulski (1979) but negative correlations were reported for other AAs (Eppendorfer and Bille, 1974; Holt and Sosulski, 1979) when these AAs are expressed as g per 16g N. Mosse *et al.* (1987) observed strong positive correlations for methionine and cystine, the present study and those of Holt and Sosulski (1979) and Evans and Boulter (1980) showed negative correlations. The negative correlation between the concentration of lysine and protein content was not significant in this study as previously reported by Eppendorfer and Bille (1974).

Figure 3 reveals that there was a good linear relationship between protein content and AA concentrations as percent of DM for arginine, lysine, leucine, valine and histidine indicating that it is possible to predict the concentrations of these AAs from protein content. The relationship is less linear for methionine and cystine. It is apparent from figure 4 that only arginine has a linear relationship with protein content while others showed non-linear relationships. This relationship is independent of genotype, environment or agricultural conditions for a particular AA ( Mosse and Baudet, 1983; Mosse *et al*, 1987).

Eppendorfer and Bille (1974) and Holt and Sosulski (1979) developed regression equations to predict AA concentrations from total N of Lysima and Century field peas respectively. The prediction equations we generated for Bohatyr from this study are similar to those of Lysima and Century for most AAs.

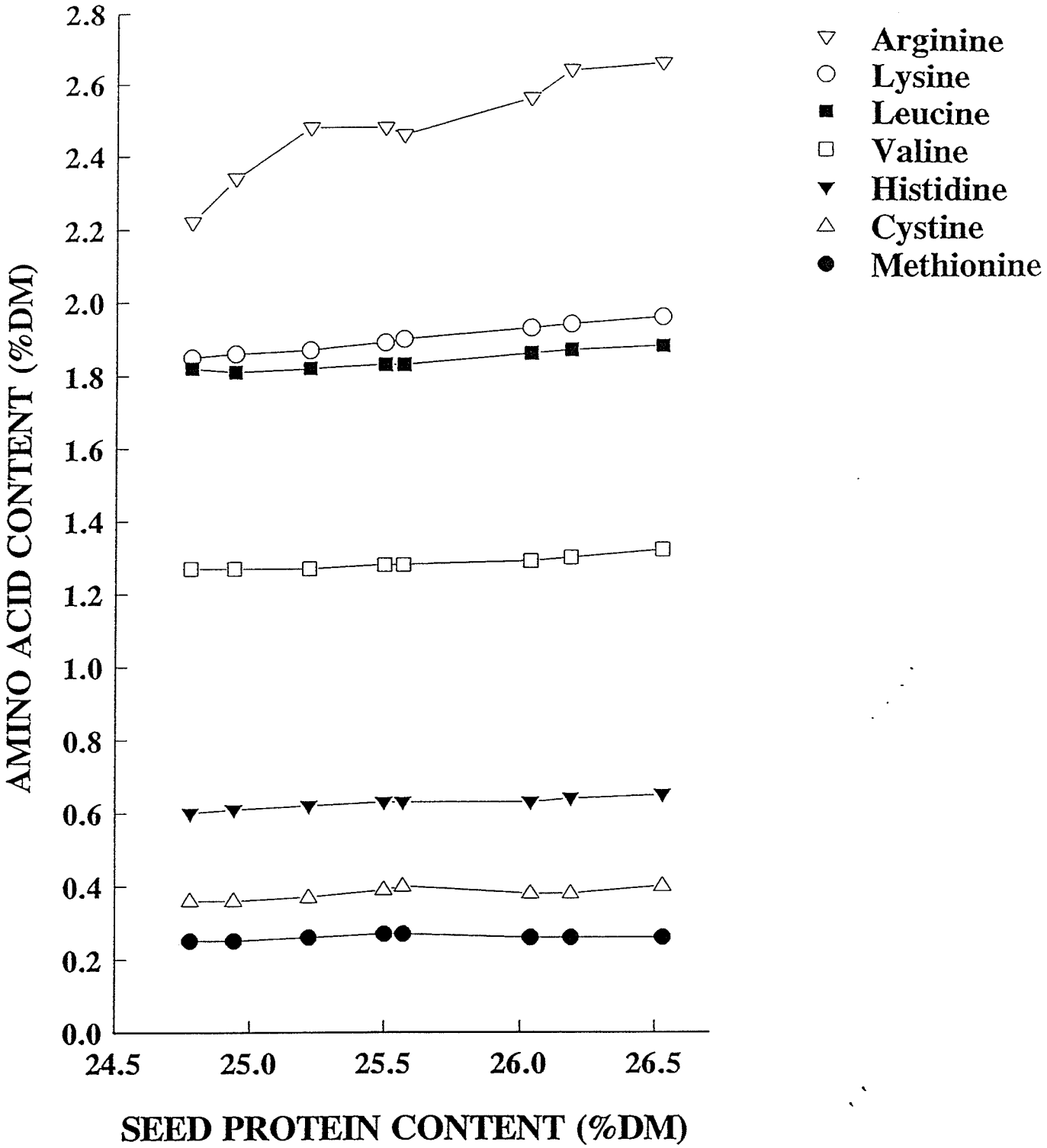


**Fig3. Relationship between basic amino acid content on a dry matter basis and seed protein content of field peas (Location 1 data).**

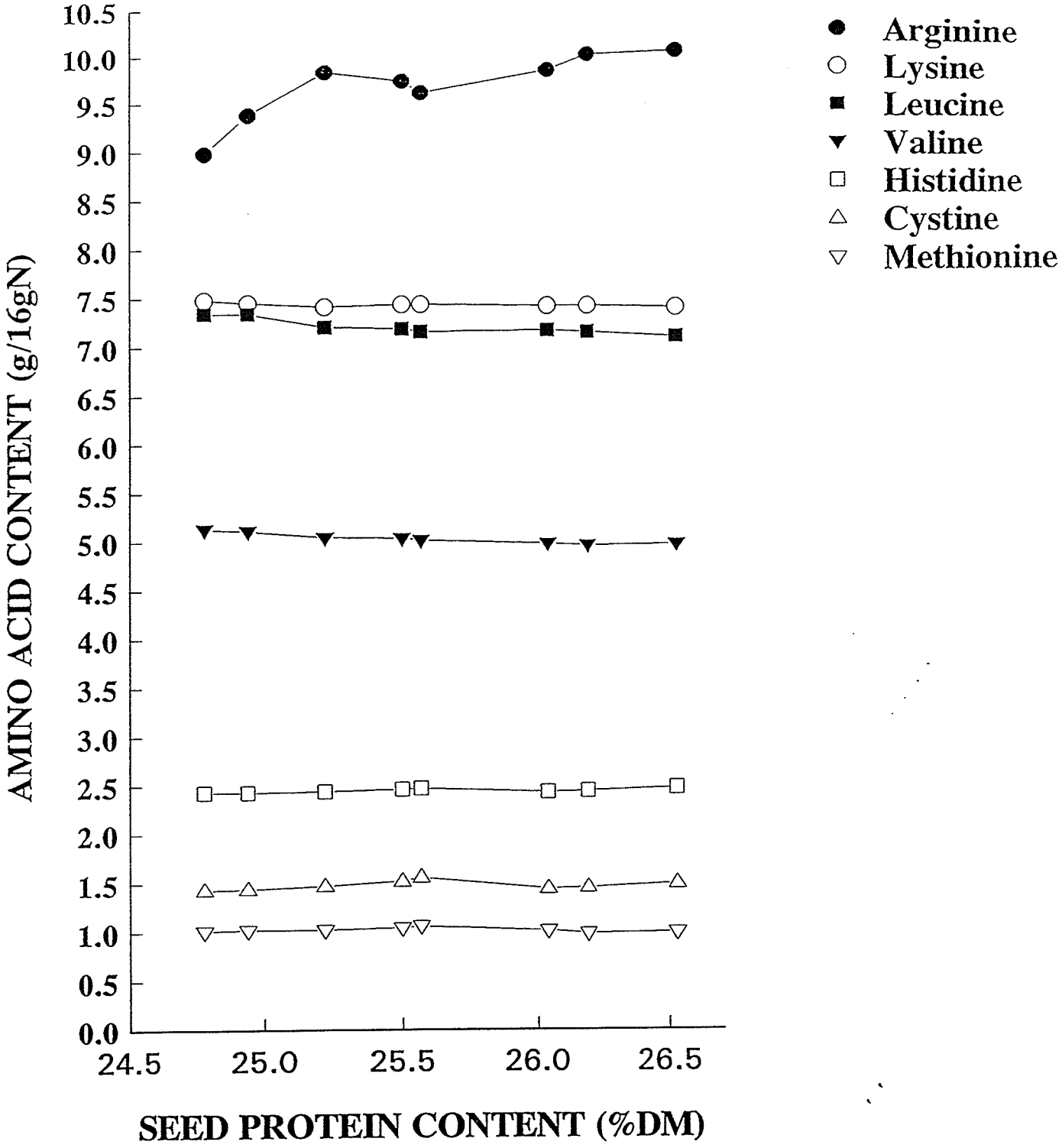


**Fig4. Relationship between basic amino acid content on a crude protein basis (g per 16g N) and seed protein content of field peas (Location 1 data).**

In conclusion, location and N application had significant effects on seed protein content and AA composition of field peas. While seed protein content increased with increasing level of N application, the conentrations of essential AAs except for arginine decreased. It appears that N application has little or no influence on the composition of essential AAs in field peas. The regression equations developed from this study could be used to predict the concentrations of AAs (except for methionine and cystine) for the cultivar Bohatyr once the protein content is known.

# CHAPTER FOUR

## USE OF PEAS IN BROILER AND LAYING HEN DIETS

Chapter four consists of three manuscripts, manuscripts 3, 4 & 5. The use of peas in broiler chicken diets was discussed in manuscript 3; the use of pea chips, pea products derived from whole peas during air classification into starch fractions in broiler diets was discussed in manuscript 4 while in manuscript 5, the use of peas in laying hen diets was discussed.

## MANUSCRIPT 3

# THE EVALUATION AND ENHANCEMENT OF THE NUTRITIVE VALUE OF YELLOW-, GREEN- AND BROWN-SEEDED PEA CULTIVARS FOR UNPELLETED DIETS GIVEN TO BROILER CHICKENS

Published in:

Animal Feed Science and Technology,

1996, **in press**

**ABSTRACT** The evaluation and possible enhancement of the feeding value for broiler chickens of yellow-, green- and brown-seeded pea cultivars were studied in three experiments. In the first experiment, bioavailable energy ($AME_n$), apparent protein digestibility (APD) and starch digestibility were determined. Dietary $AME_n$, APD and starch digestibility values were decreased ($P \leq 0.05$) with 500 g/kg inclusion of peas. The $AME_n$ and starch digestibility were similar ($P \geq 0.05$) in yellow- and green-seeded cultivars but lower ($P \leq 0.05$) in the brown-seeded cultivar. Significant differences ($P \leq 0.05$) were found between the cultivars in APD. The effects of feeding 0, 100, 200 and 400 g/kg of these cultivars on the performance of broiler chicks were examined in the second experiment. Concurrently the possibility of alleviating the detrimental effects of antinutritional factors in peas by providing excess (115% of NRC requirements) crude protein (CP) and essential amino acids (EAAs) to 400 g/kg pea-based diets was evaluated. The inclusion of up to 200 g/kg of peas did not affect weight gains but feed conversion ratio (FCR) was reduced ($P \leq 0.05$) only for the diet containing the brown-seeded peas. When peas comprised 400 g/kg of a diet fed to broiler chicks, weight gains and FCR were depressed ($P \leq 0.05$). However, this depression was alleviated by supplying excess CP and EAAs to these diets. Feed consumption was not affected by dietary inclusion of peas. The influence of adding pectinase only or in combination with protease to diets in which peas constituted a major dietary protein source was investigated in experiment 3. Supplementation of diets containing 800 g/kg peas with pectinase alone increased ($P \leq 0.05$) weight gains by 7.3 percentage units, above non-supplemented diets. Feed consumption was also improved ($P \leq 0.05$) by the same magnitude. However, feed

conversion was not affected. There was no further response observed by adding protease to these diets. It is concluded that: broiler chicks can tolerate up to 200 g/kg peas in their diets, with satisfactory performance at 400 g/kg provided CP and EAAs are supplied at 15% in excess of the NRC requirements and addition of pectinase to pea-based diets improved weight gains and feed consumption.

**Key words:** Pea cultivar, enzyme addition, nutritive value, broiler chicken,

## INTRODUCTION

Peas (*Pisum sativum L*) are legume crops which are characterized by relatively high contents of crude protein (CP) and metabolizable energy. The reported CP and metabolizable energy contents range from 160 to 320 g/kg (Marquardt and Bell, 1988; Savage and Deo, 1989; Igbasan *et al.*, 1994) and from 8.2 to 12.3 MJ/kg (Savage and Deo, 1989; Conan and Carre 1989; Igbasan *et al.*, 1994), respectively.

Despite the nutritional quality, peas remain an under-exploited feedstuff for poultry feeding. The use of peas as partial replacement of soybean meal and wheat/corn may reduce feed cost and provide a stable market for peas.

Conflicting results have been published on the acceptable levels of raw peas in the diets for growing chickens. Brenes *et al.* (1989) reported that chicks can be grown with diets containing 800g peas per kg. More recently, the same authors (Brenes *et al.*, 1993)

demonstrated that satisfactory growth performance of chicks could be obtained at 480 g peas/kg diet. However, Moran *et al.*, (1968) reported lower acceptable dietary levels of peas in their studies. These authors encountered significant depression in chick growth and feed utilization when peas were fed at 350 g/kg diet indicating that chicks could not tolerate high levels of peas in their diets as suggested by Brenes *et al.* (1989 and 1993).

The presence of pancreatic protease inhibitors in peas has been reported (Griffiths, 1984). Pancreatic proteases are rich in methionine and cystine and an inhibition of these enzymes may result in the diversion of methionine and cystine from synthesis of body tissues to additional production of pancreatic enzymes (Savage and Deo, 1989). This would further put stress on the limited methionine and cystine contents of peas. Supplemental amino acids may help to alleviate this problem.

Pea seeds, like other legumes, contain non-digestible and anti-nutrient digestion polysaccharides. Pectins/pectic substances form substantial amounts of the polysaccharides found in peas (26-55% of cell wall material from pea cotyledons and 16.8% from hulls) (Reichert 1981; Brillouet and Carre, 1983) and most are very water soluble (Annison, 1993). Almost all water-soluble polysaccharides produce viscous solutions (Oakenfull, 1993) thereby increasing digesta viscosity and reducing nutrient utilization (Cleophas *et al.*, 1995). According to Annison and Choct (1993), pectins are susceptible to endo-galactouranase cleavage. However, these polysaccharides are highly branched and this makes the backbone not readily accessible for endo-hydrolyzing enzymes. Therefore an exogenous enzyme like pectinase is needed to hydrolyze the substituents on the backbone.

Reports on the use of enzymes in legume containing diets are few. Anderson and Warnick (1964) showed no beneficial effects of supplementing soybean containing diets with enzyme. Castanon and Marquardt (1989) supplemented diets containing field beans (*Vicia faba*) with enzyme preparations and observed no major improvement in chick performance. Recently, Brenes *et al.* (1993) examined the effects of enzyme supplementation on the nutritional value of whole and dehulled peas and recorded no improvement in chick performance. Among the enzymes used by these authors are polysaccharidase, cellulase, protease and hemicellulase. There is no documented report to show whether addition of pectinase to pea-based diets would have any beneficial effect on broiler performance.

The present study had a twofold objective. One was to further establish the replacement value of peas in broiler chick diets. A comparison of the performance of chicks fed yellow-, green- and brown-seeded pea cultivars which are varying in nutrient composition will provide a basis for establishing this replacement value. The second was to investigate whether pectinase alone or in combination with protease would improve chick performance when added to diets in which peas constitute a major source of dietary protein.

## MATERIALS AND METHODS

### *Plant Materials*

The cultivars (smooth genotypes) evaluated were Impala (yellow), Radley (green) and Sirius (brown), obtained from three different locations in Manitoba, Canada. Impala

peas were supplied by Fisher Feeds, Dauphin, Radley by Roy Legumex, St. Jean and Sirius from a local pea grower in Brandon. Samples were analyzed (Table 21) for chemical composition prior to animal experimentation.

***Animal Management and Experimental Design***

One-day-old male broiler chicks were purchased from a commercial hatchery and housed in Jamesway battery brooders[1] for 3 or 4 days prior to the commencement of the experiments. During this period, the birds were fed commercial chick starter crumbles containing 210 g/kg CP (N x 6.25). To start each experiment birds were fasted for 4 h, sorted into 5 weight groups from which they were randomly assigned to each experimental pen in electrically-heated Petersime battery brooders[2].

Feed in mash form, water and light were provided continuously throughout the experiment. The temperature in the brooders was regulated to be 35 C and this was decreased by 3 C per week. The birds were weighed and feed consumption was determined on weekly basis. Before each weighing, the birds were starved for 4 h to ensure a consistent gut fill among all birds. Mean weight gain, feed consumption and feed conversion ratio (FCR) were used to determine the performance of birds.

A completely randomized design was used in experiments 1 and 2. In experiment 3, the same design was used but with a 3 x 3 factorial arrangement of treatments.

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[1]James Mfg. Co., Mount Joy, PA.

[2]Petersime Incubator Co., Gettysburg, OH 45328.

TABLE 21. Chemical composition (g/kg) of Pea cultivars.

| Parameters | Peas | | |
|---|---|---|---|
| | Impala | Radley | Sirius |
| Coat colour | Yellow | Green | Brown |
| Dry matter | 887.3 | 873.9 | 870.7 |
| Crude protein | 245.2 | 232.2 | 231.9 |
| Ash | 30.6 | 25.8 | 29.5 |
| Starch | 400.8 | 425.1 | 434.0 |
| Fat | 14.3 | 20.1 | 12.4 |
| Dietary fibre[1] | 192.3 | 192.5 | 223.1 |
| Calcium | 1.3 | 0.7 | 0.8 |
| Phosphorus | 4.3 | 4.4 | 4.4 |
| Tannin[2] | <1.0 | <1.0 | 41.0 |
| Amino acid, g per 16 g N | | | |
| Alanine | 4.2 | 4.4 | 4.4 |
| Arginine | 9.3 | 8.6 | 8.5 |
| Aspartic acid | 11.5 | 11.8 | 11.9 |
| Cystine | 1.5 | 1.6 | 1.7 |
| Glutamic acid | 16.8 | 16.3 | 16.6 |
| Glycine | 4.3 | 4.4 | 4.4 |
| Histidine | 2.4 | 2.8 | 2.4 |
| Isoleucine | 4.5 | 4.6 | 4.6 |
| Leucine | 7.3 | 7.2 | 7.0 |
| Lysine | 7.2 | 7.5 | 7.4 |
| Methionine | 1.2 | 0.9 | 0.9 |
| Phenylalanine | 4.9 | 4.8 | 4.8 |
| Proline | 4.3 | 4.4 | 4.3 |
| Serine | 4.8 | 4.8 | 4.9 |
| Threonine | 3.7 | 3.9 | 3.9 |
| Tyrosine | 3.4 | 3.7 | 3.3 |
| Valine | 4.8 | 4.7 | 4.8 |

[1] Sum of neutral detergent fibre, detergent-soluble non-starch polysaccharides, lignin and associated polyphenols, cell wall protein and minerals; [2] Tannin content was based on catechin equivalents and was detected only in pea hulls.
The values reported for crude protein, ash and tannin were not corrected for the fraction of cell wall protein, cell wall minerals and polyphenols present in the dietary fibre.

Experiment 1 was conducted for a period of 10 d while experiments 2 and 3 lasted for 14 d.

***Experiment 1***

This experiment was conducted to determine bioavailable energy ($AME_n$), apparent protein and starch digestibilities in peas. A total of 180 male broiler chicks was randomly assigned to 5 dietary treatments. Each diet was replicated with 6 pens of 6 birds per pen.

The conventional total excreta collection procedure outlined by Mollah *et al.* (1983) was used in the present study but with some modifications. A complete basal diet containing corn and soybean meal was formulated (Table 22) as a reference diet. The test ingredients (Peas) were substituted for 500 g/kg of the basal diet. Vitamin and mineral supplements were added to all diets at 15 g/kg. The final mixing proportions (g/kg) for all diets were as follows: basal diet - 485.0 g, peas - 500.0 g and vitamin and mineral mixes - 15.0 g. Diet samples were collected for laboratory analysis. All ingredients including peas were ground to pass through a screen size of 2 mm.

The 3 d balance period was initiated after the chicks had been on the test diets for 7 d. Excreta were collected daily during the balance period and immediately stored at -18 C. The lyophilized excreta samples were equilibrated at ambient temperature for 24 h before they were weighed and finely ground for laboratory analysis.

***Experiment 2***

In this experiment, the replacement value of yellow-, green- and brown-seeded

TABLE 22. Composition and chemical analysis of experimental diet, Experiment 1.

| Ingredients | Composition, g/kg |
|---|---|
| Corn | 601.6 |
| Soybean meal (48%) | 350.0 |
| Limestone | 19.0 |
| Biophos[1] | 13.5 |
| DL-Methionine | 0.9 |
| Total[2] | 985 |
| | |
| Chemical analysis, g/kg | |
| Dry matter | 902.9 |
| Crude protein | 231.6 |
| Energy (AME), MJ/kg | 13.1 |
| Starch | 456.1 |
| Lysine | 12.1 |
| Methionine | 4.8 |
| Met. + Cys. | 8.7 |
| Calcium | 10.9 |
| Avail. P. | 4.6 |

[1] Biophos: 180g Ca and 210g P per kg

[2] Vitamin and mineral supplements were added to the basal and other diets at 15g $kg^{-1}$. The final mixing proportions (g/kg) were: Basal diet - 485.0, Peas - 500.0, vitamin mix[a] - 10.0, and mineral mix[b] - 5.0g.

[a] Amount supplied per kilogram diet: Vitamin A, 8250 IU; Cholecalciferol, 991 IU; Vitamin E, 11.0 IU; Vitamin B12, 11.5 μg; Vitamin K, 1.1 mg; Riboflavin, 5.5 mg; Ca-pantothenate, 11.0 mg; Niacin, 53.0 mg; Choline chloride, 1020 mg; Folic acid, 0.75 mg; Biotin, 0.25 mg; Delaquin, 125.0 mg; Methionine, 500 mg.

[b] Amount supplied per kilogram diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg I, 0.8 mg.

ME = Metabolizable energy.

peas in broiler chick diets was evaluated. Also evaluated in the same study was the possibility of alleviating the detrimental effects of anti-nutritional factors in peas by providing excess CP and essential amino acids (EAAs) to pea-based diets.

Fourteen experimental diets (Table 23) were formulated. Diet 1 (control diet) was a practical wheat-soybean meal diet. In diets 2 to 10, the peas were added to a final concentration of 100, 200 and 400 g/kg. Diets 12 and 14 were formulated to contain excess (115% of NRC, 1994 requirements) supply of CP and EAAs while diets 11 and 13 contained marginal (85% of NRC, 1994 requirements) supply of these nutrients in yellow- and brown-seeded pea diets respectively. The yellow- and brown-seeded peas were used to formulate diets 11 to 14 because the yellow is tannin-free and widely grown in Western Canada and the brown contained an appreciable quantity of tannin. The inclusion level of peas in these diets was 400 g/kg. Diets 1 to 14 were balanced to be isoenergetic but only diets 1 to 10 were isonitrogenous. The EAA contents in all diets were formulated to be equivalent on the basis of CP.

A total of three hundred and thirty six 3 d-old male broiler chicks were used. The birds were randomly assigned to 84 electrically heated battery cages with 4 birds per cage and 6 cages per dietary treatment.

***Experiment 3***

This experiment was designed to study the effect of enzyme supplementation on the feeding value of peas. The crude enzyme preparations investigated were: pectinase and

TABLE 23. Composition and calculated analysis (g/kg) of diets based on peas, Experiment 2.

| Ingredients | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Impala[1] | - | 100 | 200 | 400 | - | - | - | - | - | - | 400 | 400 | - | - |
| Radley[1] | - | - | - | - | 100 | 200 | 400 | - | - | - | - | - | - | - |
| Sirius[1] | - | - | - | - | - | - | - | 100 | 200 | 400 | - | - | 400 | 400 |
| Wheat | 674.6 | 604.1 | 530 | 345.9 | 602.9 | 521.2 | 340.6 | 590 | 508.4 | 304.9 | 403.6 | 342 | 322.3 | 267.1 |
| Soybean | 230 | 188.8 | 150 | 117.9 | 190.8 | 161.9 | 127 | 190.8 | 145.7 | 108.2 | 51.1 | 77.0 | 94.0 | 106.8 |
| Limestone | 19.5 | 19 | 19 | 18.5 | 19 | 19 | 18.5 | 19 | 19 | 18.5 | 18.5 | 18 | 18.5 | 18.5 |
| Biophos | 13.7 | 13.3 | 13.3 | 12.9 | 13.3 | 13.3 | 12.9 | 13.3 | 13.3 | 12.5 | 13.9 | 11 | 12.5 | 11.1 |
| Vitamin[2] | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Mineral[3] | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| DL-Met. | 1.7 | 1.6 | 1.5 | 1.5 | 1.6 | 1.7 | 1.8 | 1.6 | 1.4 | 1.4 | 1.4 | 1 | 1.7 | 1.1 |
| L-Lysine | 1.5 | 0.8 | - | - | 0.6 | - | - | 0.8 | - | - | - | - | - | - |
| Casein | - | 9.0 | 17.0 | 15.5 | 10.0 | 15.4 | 17.4 | 12.5 | 27.5 | 36 | - | 80 | - | 86.3 |
| Alphacel[4] | - | - | - | - | - | - | - | - | - | - | 18 | - | 18 | - |
| Vegetable oil | 44 | 48.4 | 54.2 | 72.8 | 46.8 | 52.5 | 66.8 | 57 | 69.7 | 103.5 | 78.5 | 56 | 118 | 94.1 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Calculated analysis, g/kg | | | | | | | | | | | | | | |
| Protein | 228.9 | 229.1 | 228.6 | 228.9 | 229.1 | 228.6 | 228.6 | 229.1 | 229 | 229.1 | 195.5 | 264.1 | 195.4 | 264.3 |
| AME[5] | 12.9 | 12.9 | 12.9 | 13 | 12.9 | 13 | 13 | 12.9 | 12.9 | 13 | 13 | 13 | 13 | 13 |
| Lysine | 11 | 11.1 | 11.1 | 12.3 | 11.1 | 11.4 | 13 | 11.2 | 11.3 | 12.7 | 9.3 | 15.7 | 9.7 | 16.2 |
| Met. | 5 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 5.1 | 4.3 | 6.1 | 4.3 | 6.2 |
| Met .+ Cys. | 8.5 | 8.4 | 8.3 | 8.2 | 8.4 | 8.4 | 8.3 | 8.4 | 8.2 | 8.1 | 6.9 | 9 | 7 | 9 |
| Calcium | 10.7 | 10.5 | 10.6 | 10.4 | 10.4 | 10.4 | 10.2 | 10.5 | 10.5 | 10.2 | 10.4 | 10.2 | 10.1 | 10.3 |
| Avail. P | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.4 | 4.5 |

[1] Peas; [2, 3] Amount supplied is the same as Table 22; [4] Composed of finely ground cellulose (non-nutritive); [5] Apparent metabolizable energy, MJ/kg.
Biophos: 180 g Ca and 210 g P/kg.

protease. They were supplied by Finnfeeds International[3]. They contained 3,500 U/g pectinase and 100,000 U/g protease activities as determined by the manufacturer.

Two hundred and seventy broiler chicks that were 4 d of age were randomly distributed among 9 dietary treatments arranged as 3 x 3 factorial. Each diet was replicated with 6 pens of 5 birds per pen. The dietary treatments were: (1) Impala (800 g/kg ) pea diet (Basal 1); (2) Basal 1 plus pectinase; (3) Basal 1 plus pectinase plus protease; (4) Radley (800 g/kg) pea diet (Basal 2); (5) Basal 2 plus pectinase; (6) Basal 2 plus pectinase plus protease; (7) Sirius (800 g/kg) pea diet (Basal 3); (8) Basal 3 plus pectinase and (9) Basal 3 plus pectinase plus protease.

The 3 basal diets (Table 24) were formulated to contain low metabolizable energy and CP. With the exception of energy and CP contents, all other nutrients were formulated to meet minimum (NRC, 1994) requirements. Enzymes were introduced into the basal diets as premixes which were prepared with 500 g subsamples from the respective basal diets. Levels of enzyme supplementation were chosen according to the manufacturer's recommendations and these were: 50 U/kg diet for pectinase and 7,500 U/kg diet for protease.

***Chemical Analyses***

Prior to diet formulation, detailed chemical analyses were performed on pea samples. They were analyzed for dry matter (DM), CP, dietary fibre, ash, fat (ether

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[3]Finnfeeds International Ltd.,Marlborough, Wiltshire, UK.

TABLE 24. Composition and calculated analysis (g/kg) of basal diets, Experiment 3.

| Ingredients | Impala, basal 1 | Radley, basal 2 | Sirius, basal 3 |
|---|---|---|---|
| Peas | 800 | 800 | 800 |
| Corn | 84 | 94.2 | 55 |
| Soybean | - | 5 | 10 |
| Limestone | 17 | 18 | 18 |
| Biophos | 11 | 11 | 11 |
| DL-Methionine | 1.8 | 2.5 | 2.5 |
| Vitamin mix[1] | 10 | 10 | 10. 0 |
| Mineral mix[2] | 5 | 5 | 5 |
| Vegetable oil | 41.2 | 26 | 88.5 |
| Alphacel[3] | 30 | 28.3 | - |
| Total | 1000 | 1000 | 1000 |
| | | | |
| Calculated analysis, g/kg | | | |
| Protein | 205.9 | 199.1 | 197.6 |
| AME, MJ/kg | 11.1 | 11.1 | 10.9 |
| Lysine | 12.5 | 13.2 | 13 |
| Met. | 4.9 | 4.8 | 5 |
| Met. + Cys. | 7.6 | 7.6 | 7.5 |
| Calcium | 9.5 | 9.4 | 9.5 |
| Avail. P. | 4.2 | 4.2 | 4.2 |

[1,2] Amount supplied is the same as Table 22. [3] Composed of finely ground cellulose (non-nutritive). Biophos = 180 g Ca and 210 g P/kg.

extract), calcium, phosphorus, starch, tannin and amino acid (AA) contents. Feed and excreta samples obtained from experiment 1 were also analyzed for DM, nitrogen, gross energy and AA contents.

The DM, ash and ether extract were analyzed by standard methods published by the Association of Official Analytical Chemists (AOAC, 1984). Calcium and total phosphorus were determined according to procedures of the Association of Official Analytical Chemists (AOAC, 1990) and Analytical Methods for Atomic Absorption Spectrophotometry (Perkin-Elmer Corporation, 1973).

The analysis of neutral detergent fibre (NDF) by classical procedure is widely used to estimate fibre contents of feedstuffs for monogastric animals. However, the method understimates dietary fibre because pectins and gums which are included in dietary fibre are solubilized during the NDF determination (Bailey *et al.*, 1978). As a result, an alternative method described by Slominski *et al.* (1994) was used to estimate the dietary fibre content of peas. In this method, dietary fibre content was taken as the sum of NDF and detergent-soluble non-starch polysaccharides (NSP). The NDF component was determined using a refluxing apparatus[4] according to a procedure outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with the addition of α-amylase enzyme (Termamyl)[5]. The NSP contents of pea samples and NDF residues were quantified by gas liquid chromatography using the procedure described by Englyst

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[4]Laboratory construction Co., Kansas City, MO.

[5]Novo Nordisk A/S, Bagsvaerd, Denmark.

and Cummings (1984 and 1988) as modified by Slominski and Campbell (1990). Detergent-soluble NSP was calculated by difference (sample NSP minus NSP present in NDF residue). Cell wall protein and ash contents present in NDF residues were also determined and the value for lignin and associated polyphenols was calculated as [NDF - (NSP + protein + ash)]. Starch was obtained by difference between total glucose and NSP glucose. The method used to determine NSP was also used to measure total glucose, except that the procedure was modified to exclude the enzyme hydrolysis step.

Tannin content was measured by the method of Burns (1971) as modified by Price *et al.* (1978), using the vanillin-hydrochloric acid reagent and catechin as the standard. Nitrogen content was determined by the Kjeldhal method and converted to percentage CP where necessary (for pea and feed samples) using 6.25 as a conversion factor.

The AA content was determined using the method of Andrews and Baldar (1985) employing a LKB 4151 Alpha Plus Amino acid Analyzer[6] equipped with an LKB 4029 Programmer and a 3393A Hewlett-Packard Integrator[7]. Methionine and cystine were determined using the performic oxidation method of Hirs (1967). The apparent protein digestibility (APD) in experiment 1 was calculated from the apparent mean amino acid availability. Glycine was omitted from the calculation because uric acid is degraded to glycine and ammonia during acid hydrolysis of excreta (Soares *et al.*, 1971) resulting in higher values.

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[6]LKB Biochron Ltd., Cambridge, UK.

[7]Hewlett-Packard Co., Avondale, PA.

A Parr adiabatic oxygen bomb calorimeter[8] was used to measure gross energy of feed and excreta samples. The $AME_n$ content and apparent digestibility values assigned to peas were calculated by assuming additivity of values assigned to basal and pea fractions (Experiment 1).

### *Statistical Analysis*

Data were analyzed using the general linear models procedure of the SAS® Institute, Inc. (1986). Treatment differences obtained upon statistical analyses were subjected to the Duncan multiple range test (Duncan, 1955). The $\alpha$-level for significance was $P \leq 0.05$.

## RESULTS AND DISCUSSION

### *Chemical Composition of the Peas*

The chemical composition of the peas is given in Table 21. The DM content ranged from 870.7 to 887.3 g/kg. The CP content was highest in Impala cultivar (245.2 g/kg) and similar in Radley (232.2 g/kg) and Sirius cultivars (231.9 g/kg). Dietary fibre concentration was higher in Sirius (223.1 g/kg) than in Impala (192.3 g/kg) and Radley (192.5 g/kg). The cultivars were almost devoid of fat and calcium but well provided with starch (400.8, 425.1, 434.0 g/kg for Impala, Radley and Sirius, respectively) and phosphorus (4.3, 4.4 and 4.4 g/kg for Impala, Radley and Sirius, respectively). One major

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[8]Parr Instrument Co., Moline, IL.

difference between the cultivars was the content of tannins (<1.0, <1.0 and 41.0 g/kg for Impala, Radley and Sirius, respectively). The AA contents expressed as g per 16 g N showed some considerable variations among the cultivars. They were high in lysine and leucine but low in methionine and cystine, a characteristic of grain legumes.

The chemical compositions reported here were similar to those published elsewhere (Wright *et al.*, 1984; Savage and Deo, 1989). However, the dietary fibre contents were slightly higher. Such variation in results may reflect the differences in analytical methods. The method used here accounted for other fractions of dietary fibre such as cell wall protein (19.3 - 23.2 g/kg), cell wall minerals (2.6 - 6.4 g/kg) and lignin with associated polyphenols (25.6 - 44.5 g/kg). The higher CP content in the yellow-seeded cultivar as compared to green- and brown-seeded cultivars was reflected in lower contents of starch and dietary fibre. A Similar observation was reported by Cousin (1983). High concentrations of tannin have been reported in dark-coloured pea varieties (Griffiths, 1981). The high concentrations of lysine and leucine and low concentrations of methionine and cystine could be explained in terms of the AA composition of the main storage protein globulins in peas (Gueguen and Barbot, 1988).

The chemical composition was variable among the cultivars. The variability may be related to growing conditions or inherent varietal differences (Matthews and Arthur, 1985; Ali-Khan and Youngs, 1973).

***Experiment 1***

The results of the $AME_n$, APD and starch digestibility for diets and peas are

summarized in Table 25. These results show that dietary inclusion of peas and pea type influenced dietary $AME_n$, APD and starch digestibility ($P \leq 0.05$). The dietary values obtained when Imapla and Radley were added to the diets were similar ($P \geq 0.05$) but different ($P \leq 0.05$) from those obtained when Sirius was added to the diets for all parameters. The $AME_n$ and starch digestibility were similar ($P \geq 0.05$) in Impala and Radley cultivars which were higher ($P \leq 0.05$) than for the Sirius cultivar. Significant differences ($P \leq 0.05$) were found between all cultivars for Protein digestibility (APD).

Determinations of $AME_n$, APD and starch digestibility values of pea seeds in growing chicks by Conan and Carre (1989) showed that $AME_n$, APD and starch digestibility values of diets that contained 450 g peas per kg decreased by 8.8, 3.4 and 7.7% respectively, relative to basal diet. Brenes *et al.* (1993) also reported that $AME_n$ and APD values decreased by 13.6 and 7.2% respectively, when peas were fed to growing chicks up to 500 g/kg diet. The $AME_n$, APD and starch digestibility values for peas obtained in this study are in good agreement with values reported by these authors. It was observed that the availabilities of nutrients were always lower with brown-seeded cultivar as compared with yellow- and green-seeded cultivars. Judging from their chemical compositions, a possible explanation for this might be the presence of tannins in the brown-seeded cultivar. There is overwhelming evidence in the literature to demonstrate that tannins interfere with nutrient digestion and utilization. Marquardt (1989) and Flores *et al.* (1994) reported that tannins decrease the digestibility of protein and carbohydrates as a result of the formation of insoluble enzyme-resistant complexes with tannins. The reduction in nutrient digestion may explain the lower $AME_n$ content

TABLE 25. Apparent metabolizable energy (AMEn), apparent protein digestibility (APD) and starch digestibility of diets and pea fractions, Experiment 1.

| Treatments | $AME_n$, MJ/kg | | APD, % | | Starch digestibility, % | |
|---|---|---|---|---|---|---|
| | Diet | Pea | Diet | Pea | Diet | Pea |
| Corn-soy (CS) | 13.1a | - | 86.4a | - | 96.1a | - |
| CS + MP (50:50) | 11.4b | 10.5b | 80.8b | 75.2a | 90.2b | 85.8a |
| CS + RD (50:50) | 11.6b | 10.9b | 79.6b | 72.8b | 92.2b | 88.5a |
| CS + SR (50:50) | 10.3c | 8.4c | 73.4c | 60.4c | 86.5c | 76.9b |
| SEM | 0.08 | 0.12 | 0.25 | 0.55 | 0.58 | 1.09 |

MP = Impala; RD = Radley; SR = Sirius.
abc Means in the same column with different letters differ significantly ($P \leq 0.5$).

in this cultivar. Brenes *et al.* (1993) also reported lower $AME_n$ value for the tannin-containing Maple cultivar.

The performance data obtained in this trial are shown in Table 26. Mean weight gains and FCR of broiler chicks fed the diets containing 500 g/kg of yellow-, green- and brown-seeded peas were poorer ($P \leq 0.05$) than those of birds fed the control diet. Feed consumption was essentially the same except for birds fed green-seeded peas which was lower ($P \leq 0.05$) than all other diets. However, it should be recognized that this trial was not designed to study growth performance but to determine nutrient availabilities.

***Experiment 2***

Table 27 shows mean weight gains, feed consumption and FCR for a period of 14 d of broiler chicks fed graded levels of yellow-, green- and brown-seeded pea cultivars. Presented in the same table are data to show the performance of broiler chicks fed 400 g/kg of peas under conditions of excess (115% of NRC) or marginal (85% of NRC) supply of CP and EAAs. Although there was a trend for poorer weight gains and FCR of broiler chicks fed diets containing either 100 or 200 g/kg of any of the pea cultivars the differences were not significant ($P \geq 0.05$) from those of birds fed the wheat-soy control diet. The only exception was the FCR of birds fed the diet containing 200 g/kg of brown-seeded cultivar which was significntly different ($P \leq 0.05$) from all others. Chicks fed diets containing 400 g/kg of any of the pea cultivars had reduced ($P \leq 0.05$) weight gains and FCR. The reduction was more pronounced with the brown-seeded cultivar. There was no effect of dietary inclusion of peas or that of pea type on feed

TABLE 26. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (5-12 d) fed yellow-, green- and brown-seeded peas, Experiment 1.

| Treatments | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| Corn-soy (CS) | 139.7a | 197.5a | 1.41a |
| CS + MP (50:50) | 126.6b | 203.9a | 1.60b |
| CS + RD (50:50) | 116.3c | 189.9b | 1.63b |
| CS + SR (50:50) | 113.9c | 204.2a | 1.79c |
| SEM | 1.63 | 2.45 | 0.032 |

abc Means in the same column with different letters differ significantly ($P \leq 0.05$).
MP = Impala; RD = Radley; SR = Sirius.

TABLE 27. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (3-17 d) fed graded levels of yellow- green- and brown-seeded peas, Experiment 2.

| Dietary peas inclusion rate, g/kg | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| 0 | 345.8ab | 507.9a | 1.47a |
| MP-100 | 340.7abc | 505.5a | 1.48a |
| MP-200 | 329.2abcd | 500.5a | 1.52abc |
| MP-400 | 323.7cde | 506.8a | 1.57bcde |
| RD-100 | 338.0abc | 499.5a | 1.48a |
| RD-200 | 325.5bcde | 503.3a | 1.55abcd |
| RD-400 | 313.6def | 494.8a | 1.58cd |
| SR-100 | 337.7ab | 503.7a | 1.49ab |
| SR-200 | 326.7abcde | 518.0a | 1.59de |
| SR-400 | 302.9fg | 506.7a | 1.67fg |
| MP-400, marginal[1] | 307.3efg | 502.5a | 1.64ef |
| MP-400, excess[2] | 346.9a | 512.7a | 1.48a |
| SR-400, marginal | 294.0g | 503.5a | 1.72g |
| SR-400, excess | 333.6abcd | 508.2a | 1.53abcd |
| SEM | 6.33 | 10.72 | 0.034 |

a-g Means in the same column with different letters differ significantly ($P \leq 0.05$).
MP = Impala; RD = Radley; SR = Sirius.
[1] Marginal - Contained 85% of NRC requirements for crude protein and essential amino acids for broilers.
[2] Excess - Contained 115% of NRC requirements for crude protein and essential amino acids for broilers.

consumption observed in this study.

Feeding diets containing 400 g/kg of yellow- and brown-seeded peas to broiler chicks under conditions of marginal supply of CP and EAAs significantly influenced ($P \leq 0.05$) mean weight gains and FCR. Those birds that received diets containing 400 g peas per kg formulated to meet NRC requirements (NRC 1994) for broilers had their mean weight gains reduced by 6 and 12% for yellow- and brown-seeded peas respectively, relative to those birds fed the control diet. With marginal supply of CP and EAAs, mean weight gains decreased ($P \leq 0.05$) by 11 and 15% for yellow- and brown-seeded peas, respectively. However under conditions of excess supply of CP and EAAs, mean weight gains were similar to the control diet. The FCR followed the same pattern as described for weight gains. Feed consumption was not affected by diet nutrient concentration.

The present results, showing fairly consistent growth depression when different pea cultivars were fed to broiler chicks up to 400 g/kg, are contrary to previous studies carried out by Brenes *et al.* (1989 and 1993). These authors reported that inclusion of peas into chick diets up to 800 g/kg had no detrimental effects on growth performance. In a second paper, the same authors (Brenes *et al.* 1993) showed that satisfactory growth performance of chicks could be maintained at 480 g peas per kg diet. However, our results seem in agreement with those of Moran *et al.* (1968) who found a significant depression in performance of growing chicks fed a diet containing 350 g peas per kg. Our recent experience with pea chips (Igbasan and Guenter 1996b) also revealed that even at 300 g peas per kg diet and with methionine supplementation to 120% of the NRC requirement,

broiler chicks were significantly smaller than their counterparts fed on corn-soybean diet. The presence of different levels of antinutritive factors such as protease inhibitors and polyphenolic compounds coupled with differences in nutrient contents of peas may be responsible for some of the inconsistencies among reports in the literature.

The provision of excess CP and EAAs to diets containing a high level of yellow- and brown-seeded peas markedly improved weight gain and feed conversion. This indicates that it is possible to alleviate some of the growth depressing effects of the antinutritive factors in peas by providing supplementary protein and EAAs in excess of NRC requirements. Griffiths (1981 and 1984) has documented the presence of trypsin inhibitors and condensed tannins in peas. The inactivation of trypsin in the gut by trypsin inhibitors results in inadequate digestion of protein and in the diversion of S-containing AAs from synthesis of body tissues to additional production of pancreatic enzymes (Savage and Deo, 1989). On the other hand, tannins exert their antinutritive effect by binding to proteins (Marquardt, 1989) thereby making them unavailable for host animals to use. The excess CP and EAAs provided in diets in this study were probably used to produce more trypsin or as tannin binding agents, which would diminish or eliminate the harmful effects of these antinutritive factors leading to an improvement in weight gain and feed conversion. Since our diets were not formulated on the basis of digestible amino acids of peas but rather on total amino acids, it is also possible that part of the EAAs provided in excess of NRC recommendations were used to meet the actual amino acid requirements of broiler chicks. It should be noted that the digestibilities of most EAAs in raw peas are low (Igbasan *et al.*, 1994).

*Experiment 3*

Supplementation of diets containing 800 g/kg of peas with pectinase alone supported ($P \leq 0.05$) weight gains in broiler chicks by 7.3 percentage units, above non-supplemented diets (Table 28). Feed consumption was also improved ($P \leq 0.05$) by the same magnitude. However, feed conversion was not affected ($P \geq 0.05$). The addition of a combination of pectinase and protease to these diets did not produce further response either in weight gains or in feed consumption. The lack of interaction between type of peas and enzyme observed in this study indicates that the response to enzyme was similar for all test peas.

The results of this study show that addition of pectinase to pea-based diets resulted in an improvement in weight gains and feed consumption but had no effect on feed conversion. These data are in contrast to the results reported by Brenes *et al.* (1993) who found a reduction in weight gains and feed consumption but not in feed to gain ratio when enzymes were added to diets containing whole or dehulled peas. Such discrepancies in results could be explained by difference in enzyme preparations used in both studies. The enzyme preparations used in their study contained cellulase, amylase and protease. The fact that enzyme addition had no effect on feed conversion indicates that the improvement in feed consumption observed here was mainly attributable to an increase in intestinal passage rate which stimulated appetite. This improvement in feed consumption resulted in an overall improvement in weight gain.

The present data suggests that peas could serve as protein and energy supplements in broiler diets, but inclusion level should be restricted to 200 g/kg diet. Satisfactory

TABLE 28. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (4-18 d) fed raw peas (800 g/kg) supplemented with enzymes, Experiment 3.

| Treatments | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| PEAS | | | |
| Impala | 338.6a | 592.5a | 1.75a |
| Radley | 327.8a | 575.2a | 1.76a |
| Sirius | 308.4b | 571.4a | 1.87b |
| ENZYME | | | |
| No enzyme | 308.4b | 553.6b | 1.80a |
| + Pect[1]. | 332.1a | 587.0a | 1.78a |
| + Pect. + Prot[2]. | 334.4a | 598.5a | 1.79a |
| SEM | 3.97 | 8.88 | 0.031 |

[1] Pectinase enzyme; [2] Protease enzyme.
ab Means in the same column under peas or enzyme with different letters differ significantly ($P \leq 0.05$).
There was no interaction ($P \geq 0.05$) between peas and enzymes.

performance could be maintained at 400 g/kg provided crude protein and essential amino acids are supplied in excess (115%) of the NRC requirements.

The use of pectinase to enhance the nutritive value of peas could be beneficial as evidenced from the improvement in weight gains and feed consumption, but more research is required in this area.

## MANUSCRIPT 4

# THE FEEDING VALUE FOR BROILER CHICKENS OF PEA CHIPS DERIVED FROM MILLED PEAS (*PISUM SATIVUM* L.) DURING AIR CLASSIFICATION INTO STARCH FRACTIONS

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**ABSTACT** Two pea products, yellow pea chips (YPC) and green pea chips (GPC) that were derived from milled peas during air classification into pea starch fractions were evaluated for their nutritional value in three experiments (experiments 4, 5, & 6). The products were found to contain 298.3 and 281.3 g/kg crude protein, 7.28 and 7.10 g lysine per 16g N and 1.05 and 0.94 g methionine per 16 g N for YPC and GPC respectively. The apparent metabolizable energy value of YPC was higher ($P \leq 0.05$) than that of GPC, 11.50 vs 11.28 MJ/kg, respectively. Similar result was also obtained with starch digestibility, 81.6 vs 77.4% ($P \leq 0.05$), however apparent protein digestibility was similar ($P \geq 0.05$) (experiment 4). In experiment 5, YPC or GPC replaced corn and soybean meal at 0, 150, 300 and 450 g/kg in broiler chick diets. Weight gain and feed conversion ratio (FCR) were similar ($P > 0.01$) for birds fed the 150 g/kg of YPC (397.2 g, 1.41) or GPC (390.4 g, 1.42) diet and the control (403.1 g, 1.38) diet. At 300 and 450 g/kg inclusion levels, weight gain (377.9, 345.5 g; 362.9, 306.6 g) and FCR (1.48, 1.51; 1.47, 1.61) decreased significantly ($P < 0.01$) for both products relative to the control (403.1 g, 1.38). Feed consumption decreased with increasing levels of pea chips in the diets but the effect was significant ($P < 0.01$) only in those diets containing 450 g/kg. In experiment 6, diets containing 300 g/kg of YPC or GPC diet were supplemented with two levels of DL-methionine to 100% and 120% of the NRC requirements. The performance of these birds was still lower ($P <0.01$) than that of birds fed the control diet. The fact that these pea by-products at 300 g/kg inclusion level with methionine supplementation were unable to sustain broiler performance equal to birds fed conventional corn-soy diet, suggests that they should not be fed to broiler chicks in excess

of 150 g/kg.

**Key words:** Air classification, pea chips, broiler chick, methionine.

## INTRODUCTION

The increasing cost and demand for protein has stimulated considerable research interest in the development of alternative sources of proteins for livestock feeding. Field peas (*Pisum sativum L.*) with their comparatively high content of protein and essential amino acids, lysine and leucine (Savage and Deo, 1989; Gatel and Grosjean, 1990) and metabolizable energy (Savage and Deo, 1989; Igbasan, *et al.,* 1994) have received much attention.

While the production of this crop in Western Canada has increased dramatically over the last few years (Ali-Khan and Zimmer, 1989; Castel *et al.* 1996) its use in poultry feeding is very limited. Over 80% of the Canadian field pea production is exported mainly to markets in European countries (Ali-Khan and Zimmer, 1989; Slinkard, 1994). Poultry diets in Western Canada are based on wheat or corn with imported soybean meal as the major protein supplement. The use of pea products as partial replacement of soybean meal may help to reduce feed costs.

With the application of impact-milling and air classification technology, field peas are now being processed into pea hull, pea starch and pea protein concentrate to enhance their utilization in the food industries and to increase domestic consumption. The fractionation of pea flours into pea starch and pea protein concentrate by air classifier

usually results in unavoidable losses of portions of the flours in the collection systems (Tyler *et al.,* 1981; Wright *et al.*, 1984). These portions vary in particle size, between fine and coarse, depending on the stage of fractionation. Normally, the coarse portions are obtained at the initial stages of milling and classification but as these processes continue, the fine portions are produced. At the processing plants, these products are grouped into "superfines", "fines" and "pea chips" and they constitute industrial wastes because currently there is no market for them (Both, 1993, Process Manager, Woodstone Foods Corporation, Personal communication). Because of the powdery nature of "superfines" and "fines", only pea chips could have practical application in poultry feeding. Pea chips are coarse and uniform in particle size.

The nutritional composition and feeding value of air-classified field pea fractions have been well documented (Bell and Youngs, 1970; Bhatty and Christison, 1984). However, there is a lack of information on the composition and feeding value of pea chips. The objective of this study is to evaluate the nutritional value of pea chips in diets for broiler chickens.

## MATERIALS AND METHODS

### *General Procedures*

Two types of pea chips, yellow pea chips (YPC) and green pea chips (GPC), were obtained from Woodstone Foods Corporation[1]. These pea chips were analyzed for

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[1]Woodstone Foods Corporation, Portage La Prairie, manitoba, Canada

TABLE 29. Chemical composition of pea products, g/kg.

| Parameter | Pea product | |
|---|---|---|
| | Yellow pea chips, YPC | Green pea chips, GPC |
| Dry matter | 876.9 | 908.0 |
| Crude protein | 298.3 | 282.2 |
| Fat | 22.3 | 15.3 |
| NDF | 131.8 | 134.5 |
| Ash | 40.9 | 30.5 |
| Calcium | 05.5 | 4.0 |
| Phosphorus | 0.4 | 0.5 |
| Starch | 383.7 | 431.3 |
| Amino acid, g per 16 g N | | |
| Alanine | 4.21 | 4.12 |
| Arginine | 9.66 | 10.83 |
| Aspartic acid | 11.98 | 11.55 |
| Cystine | 1.34 | 1.25 |
| Glutamic acid | 17.08 | 16.67 |
| Glycine | 4.21 | 4.14 |
| Histidine | 2.48 | 2.44 |
| Isoleucine | 4.66 | 4.22 |
| Leucine | 7.24 | 6.94 |
| Lysine | 7.28 | 7.10 |
| Methionine | 1.05 | 0.94 |
| Phenylalanine | 4.77 | 4.55 |
| Proline | 4.25 | 4.08 |
| Serine | 4.86 | 4.79 |
| Threonine | 3.75 | 4.63 |
| Tyrosine | 3.23 | 3.23 |
| Valine | 4.95 | 4.74 |

All analyses were performed in duplicate and all parameters, except dry matter were expressed as percent dry matter.

chemical composition (Table 29) before they were incorporated into experimental diets at various levels. All diets were given in mash form and water and feed were provided *ad libitum* throughout the entire experiment. One-day-old male broiler chicks were purchased from a commercial hatchery and were raised in Jamesway battery brooders[2] for 5 days. During this period they were fed commercial chick starter crumbles (210 g/kg CP). At 5 days of age, the birds were housed in Petersime battery brooders[3] and were randomly assigned to the experimental diets. Experiment 4 was conducted for a period of 16 days while experiments 5 and 6 lasted for 14 days. The birds were weighed and feed consumption was determined on weekly basis. Chick performance was measured in terms of weight gain, feed consumption and feed conversion ratio and the overall mean values were reported for each experiment on a per bird basis.

***Experiment 4***

This experiment was conducted to determine the apparent metabolizable energy ($AME_n$) content and nutrient digestibilities of YPC and GPC. A total of 90 male commercial broiler chicks were randomly assigned to three dietary treatments. Each dietary treatment was replicated with five pens of six birds each. The experiment was carried out using the conventional total collection procedure, essentially as described by Mollah *et al.* (1983) but with modifications. Birds were fed on the test diets for 2 wks

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[2]James Mfg. Co., Mount Joy, PA.

[3]Petersime Incubator Co., Gettysburg, OH.

prior to a 2 d collection period. During this 2 week period, body weight and feed consumption were recorded.

Three experimental diets were prepared. A complete basal diet containing corn and soybean meal was prepared as a reference material. The test ingredients (YPC and GPC) were used to replace the basal diet at 450 g/kg. The composition of the basal diet is presented in Table 30.

Excreta were collected daily during the balance period and immediately stored at -18 C. Excreta were freeze dried, equilibrated at ambient temperature for 24 h, weighed and ground for chemical analyses.

***Experiment 5***

This experiment was designed to evaluate the replacement value of YPC and GPC for corn and soybean meal in diets for broiler chickens. One hundred and forty male commercial broiler chicks were randomly allocated to 35 electrically heated battery pens. Each dietary treatment was replicated with five pens of four birds each and fed from 5 d to 19 days of age. Seven experimental diets were formulated to contain 0, 150, 300 and 450 g/kg diet of YPC or GPC replacing corn and soybean meal. All diets were formulated to be isoenergetic and isonitrogenous (N x 6.25) but were supplemented with only 0.05 g/kg DL-methionine. With the exception of total sulphur amino acid content, all other nutrients were formulated to either meet or slightly exceed NRC (1994) requirements for broilers. The compositions of the diets are given in Table 31.

TABLE 30. Composition and determined analysis of the basal diet used with broilers, Experiment 4.

| Ingredient | Composition, g/kg |
|---|---|
| Corn | 603 |
| Soybean meal | 338 |
| Calcium carbonate | 25 |
| Dicalcium phosphate | 18 |
| Mineral mix[1] | 5 |
| Vitamin mix[2] | 10 |
| DL-Methionine | 1 |
| | |
| Determined Analysis. g/kg | |
| Dry matter | 898.0 |
| Crude Protein, N x 6.25 | 224.8 |
| Starch | 408.7 |
| Calcium | 10.6 |
| Phosphorus | 5.0 |
| Lysine | 12.1 |
| Methionine | 5.1 |
| Methionine + Cystine | 8.5 |
| AMEn, MJ/kg, DM basis | 13.17 |

[1] Amount supplied per kg diet: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.8 mg.

[2] Amount supplied per kg diet: Vitamin A, 8250 IU; Cholecalciferol, 991 IU; Vitamin E, 11 IU; Vitamin $B_{12}$, 11.5μg; Vitamin K, 1.1 mg; Riboflavin, 5.5 mg; Ca-pantothenate, 11.0 mg; Niacin, 53 mg; Choline chloride, 1020 mg; Folic acid, 0.75 mg; Biotin, 0.25 mg; Delquin ( ethoxyquin - anti-oxidant), 125.0 mg, methionine; 500 mg

TABLE 31. Composition and calculated analysis (g/kg) of experimental diets containing pea chips and fed to growing broilers, Experiment 5.

| Ingredients | CON[1] | Yellow pea chips | | | Green pea chips | | |
|---|---|---|---|---|---|---|---|
| Peas | - | 150.00 | 300.0 | 450.0 | 150.0 | 300.0 | 450.0 |
| Corn | 518.5 | 450.5 | 382.7 | 315.7 | 440.9 | 363.6 | 286.7 |
| Soybean | 401.0 | 316.0 | 231.5 | 146.0 | 323.0 | 245.0 | 168.0 |
| Limestone | 18.0 | 19.0 | 19.5 | 20.3 | 19.0 | 20.0 | 20.0 |
| Biophos[2] | 12.5 | 11.2 | 10.0 | 8.8 | 11.1 | 9.8 | 8.5 |
| Vitamin mix[3] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral mix[4] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vegetab. oil | 35.0 | 38.3 | 41.3 | 44.2 | 41.0 | 46.6 | 51.8 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Calculated analysis, g/kg | | | | | | | |
| Protein | 230.4 | 230.2 | 230.3 | 230.1 | 230.1 | 229.9 | 230.3 |
| AME. MJ/kg | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 |
| Lysine | 13.6 | 13.6 | 13.4 | 13.3 | 13.6 | 13.5 | 13.3 |
| Methionine | 4.2 | 3.9 | 3.7 | 3.5 | 3.9 | 3.6 | 3.5 |
| Met.+Cys. | 8.1 | 7.6 | 7.1 | 6.8 | 7.6 | 7.1 | 6.7 |
| Ca. | 10.0 | 10.1 | 10.1 | 10.2 | 10.1 | 10.3 | 10.0 |
| Avail. P | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |

[1] Control diet.
[2] Biophos: 180 g Ca and 210 g P/kg
[3] Same as Table 30; [4] Same as Table 30.

*Experiment 6*

This experiment was designed to study the effect of supplementing YPC and GPC diets with methionine. One hundred male chicks of commercial broiler strain were randomly distributed among 5 dietary treatments. Each dietary treatment was replicated with 5 pens of four birds per pen and fed from 5 d to 19 d of age.

Diets containing YPC or GPC at an inclusion level of 300 g/kg diet (Table 32) were supplemented with two levels of DL-methionine to 100 and 120% of the NRC requirements (NRC, 1994). A corn/soy control diet was also formulated to meet NRC (1994) requirements for broilers. All diets were formulated to be isoenergetic and isonitrogenous (N x 6.25).

*Chemical Analyses*

Detailed chemical analyses were performed on the pea products. They were analyzed for dry matter (DM), protein (N x 6.25) neutral detergent fibre (NDF), ash, fat (ether extract), calcium, phosphorus, starch and amino acid contents. The DM, protein (N x 6.25), fat and ash were analyzed by standard methods published by the Association of Official Analytical Chemists (AOAC, 1984). The NDF was determined using a refluxing apparatus[4] as outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with the addition of α-amylase enzyme (Termanyl)[5]. Calcium and total

[4]Laboratory construction Col. Kansas City, MO.

[5]Novo Nordisk A/S, Bagsvaerd, Denmark.

TABLE 32. Composition and calculated analysis (g/kg) of experimental diets containing pea chips and fed to growing broilers, Experiment 6.

| Ingredients | CON[1] | YPC[2] | | GPC[3] | |
|---|---|---|---|---|---|
| | | YPC-100[4] | YPC-120[4] | GPC-100 | GPC-120 |
| Peas | - | 300.0 | 300.0 | 300.0 | 300.0 |
| Corn | 518.5 | 384.6 | 384.6 | 364.0 | 364.0 |
| Soybean | 400.1 | 228.9 | 227.8 | 243.3 | 242.2 |
| Limestone | 18.0 | 19.5 | 19.5 | 20.0 | 20.0 |
| Biophos[5] | 12.5 | 10.0 | 10.0 | 9.8 | 9.8 |
| DL-Methionine | 0.9 | 1.5 | 2.6 | 1.6 | 2.7 |
| Vitamin mix[6] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral mix[7] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vegetable oil | 35.0 | 40.5 | 40.5 | 46.3 | 46.3 |
| Total | 1000 | 1000 | 1000 | 1000 | 1000 |
| Calculated analysis, g/kg | | | | | |
| Protein | 230.3 | 230.2 | 230.3 | 230.2 | 230.3 |
| AME, MJ/kg | 12.8 | 12.8 | 12.8 | 12.8 | 12.8 |
| Lysine | 13.6 | 13.3 | 13.3 | 13.4 | 13.3 |
| Methionine | 5.0 | 5.0 | 6.0 | 5.0 | 6.0 |
| Met. + Cys. | 9.0 | 8.6 | 9.7 | 8.6 | 9.7 |
| Ca | 10.0 | 10.1 | 10.2 | 10.3 | 10.3 |
| Avail. P | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 |

[1] Control diet; [2] Yellow pea chips; [3] Green pea chips.
[4] 100 or 120% NRC Methionine Requirement.
[5] Same as Table 31; [6] Same as Table 30; [7] Same as Table 30.

phosphorus were determined according to procedures of the Association of Official Analytical Chemists (AOAC, 1990) and Analytical Methods for Atomic Absorption Spectrophotometry (Perkin-Elmer Corporation, 1973). The starch content for both products, diets and excreta samples was taken as the difference between the total glucose and non-starch polysaccharide (NSP) glucose. Total glucose was determined using a modified NSP determination procedure described by Englyst and Cummings (1984) but also with minor modifications (Slominski and Campbell, 1990). The enzyme hydrolysis step in the procedure was omitted after the sample had been gelatinized/solubilized in a boiling water bath for 0.5 h in 0.1M sodium acetate buffer. The total glucose and the NSP glucose were quantified by using gas-liquid chromatography.

The amino acid composition for both products, diets and excreta samples was determined as outlined by Andrews and Baldar (1985) with performic acid oxidation of cystine and methionine according to Hirs (1967). Final analysis was carried out by employing an LKB 4151 Apha Plus Amino acid Analyzer[6] equipped with an LKB 4029 Programmer and a 3393A Hewlett-Packard Integrator[7].

The apparent protein digestibilty (APD) was calculated from the weighted average of apparent digestibilities of individual amino acids. Glycine was omitted from this calculation since uric acid is degraded to glycine and ammonia during the acid hydrolysis of excreta (Soares *et al.,* 1971).

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[6]LKB Biochron Ltd., Cambridge Science Park, Cambridge, UK.

[7]Hewlett-Packard Co., Avondale, PA.

Feed and excreta samples were analysed for gross energy using a Parr adiabatic oxygen bomb calorimeter[8]. Nitrogen was determined using the Kjeldahl procedure (AOAC, 1984). The AMEn content and the apparent digestibility values assigned to the pea products were calculated by assuming additivity of values assigned to basal and pea product fractions.

### *Statistical Analysis*

All experiments were set up as completely randomized designs and data were subjected to analysis of variance using the General Linear Models (GLM) procedure of the Statistical Analysis System, Institute, Inc. programme (SAS® Institute, Inc. 1986). When analysis of variance indicated a significant treatment effect, Duncan's multiple range test (Duncan, 1955) (experiment 4) and linear contrasts (experiments 5 and 6) were used to compare treatment means.

## RESULTS AND DISCUSSION

### *Chemical Composition of Pea Products*

The composition of the two pea products used in this study is summarized in Table 29. Protein and NDF contents were similar. While GPC had a higher starch content, YPC was higher in calcium content. The starch contents, 383.7 and 431.3 g/kg

[8]Parr Instrument Co., Moline, IL.

for YPC and GPC respectively, are comparable to that reported for whole pea seeds (Savage and Deo, 1989; Gatel and Grosjean, 1990; Brenes *et al.,* 1993; Igbasan *et al.,* 1994).

The crude protein contents, 298.3 and 281.3 g/kg for YPC and GPC respectively were slightly higher than the values normally found in whole pea seeds as reported by the same authors but in good agreement with values reported for pea flours (Vose *et al.,* 1976; Tyler *et al.,* 1981; Reichert, 1981 Wright *et al.,* 1984).

The amino acid profile of the pea products showed that YPC and GPC proteins contain high concentrations of lysine and leucine and low concentrations of methionine and cystine, a characteristic of grain legumes. The YPC protein tended to contain higher content of most amino acids than GPC protein. There is also a good agreement between the amino acid composition of these products and the amino acid composition reported for pea flours by other researchers (Vose *et al.,* 1976; Leterme *et al.,* 1990). Calcium, phosphorus and NDF contents are within the range of values reported in the literature for dehulled peas (Savage and Deo, 1989).

Both YPC and GPC have chemical composition different from the chemical composition of pea starch and protein concentrates because they are derived primarily from pea flours during the process of fractionation into starch fractions. The difference in chemical composition between the products may be attributed to varietal differences. Peas are noted to contain variable chemical composition, which in most cases is a reflection of variation among varieties and environmental conditions (Ali-Khan and Youngs, 1973; Shia and Slinkard, 1977).

*Experiment 4*

The $AME_N$ value of YPC was slightly ($P \leq 0.05$) higher (11.50 vs 11.28 MJ/kg) than that of GPC (Table 33). Similar results were also obtained with starch digestibility, 81.6 vs 77.4% ($P \leq 0.05$); however, APD was similar ($P \geq 0.05$). The $AME_n$, starch digestibility and APD values of diets decreased ($P < 0.05$) with addition of pea products.

The mean starch digestibility and APD values of YPC and GPC are slightly higher than most values reported (Conan and Carre ,1989; Brenes *et al.,* 1993) for whole pea seeds. This improvement may be attributed to processing, which not only eliminates the hulls which are high in fibre content but also increases the concentration of digestible protein and starch. The processing may also disrupt endosperm cell wall structure and thickness thereby making starch granules accessible to enzyme attack. The $AME_n$ content is also higher than the values reported by the same authors and this could be related to the improvement in protein and starch digestibility.

Although not very important in this study, the growth data (Table 34) showed that there was significant difference ($P < 0.05$) in weight gain and FCR among the birds fed basal diet and either 450 g/kg of YPC or GPC. Feed consumption was lower ($P < 0.05$) for chicks fed the YPC diet.

*Experiment 5*

The effect of increasing the level of inclusion of pea products in the diet on growth, feed consumption and feed conversion ratio (FCR) of broiler chicks from 5 to 19 days of age is shown in Table 35. Substitution of YPC or GPC for corn and soybean

TABLE 33. Apparent metabolizable energy value (AMEn), apparent protein digestibility (APD) and starch digestibility of diets and pea products, Experiment 4.

| Treatments | AMEn, MJ/kg[1] | | APD, %[1] | | Starch Digestibility, %[1] | |
|---|---|---|---|---|---|---|
| | Diet | Pea product | Diet | Pea product | Diet | Pea product |
| Corn-soy basal, CS | 13.17[a] | - | 88.1[a] | - | 94.6[a] | - |
| CS +45%YPC[2] | 12.41[b] | 11.50[a] | 86.2[b] | 83.3[a] | 88.7[b] | 81.6[a] |
| CS +45%GPC[3] | 12.31[c] | 11.28[b] | 85.8[b] | 82.9[a] | 86.8[c] | 77.4[b] |
| SEM | 0.061 | 0.080 | 0.15 | 0.56 | 0.19 | 0.67 |

[1] Means obtained from five replicates.
[2] Yellow pea chips; [3] Green pea chips.
[abc] Means in the same column with different superscripts differ significantly ($P < 0.05$).

TABLE 34. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (5-19 days) fed pea products, Experiment 4.

| Treatments | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| Corn-soy basal, CS | 440.5[a] | 648.5[a] | 1.47[a] |
| CS + 45%YPC[1] | 360.7[b] | 569.8[b] | 1.58[b] |
| CS + 45%GPC[2] | 365.8[b] | 617.3[a] | 1.69[c] |
| SEM | 9.91 | 12.43 | 0.112 |

[1] Yellow pea chips; [2] Green pea chips.
[abc] Means in the same column with different superscripts differ significantly ($P < 0.05$).

TABLE 35. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (5-19 days) fed graded levels of pea products without methionine supplementation, Experiment 5.

| Treatments | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| 1 Corn-soy | 403.1 | 560.4 | 1.38 |
| 2 15% YPC[1] | 397.2 | 563.4 | 1.41 |
| 3 30% YPC | 377.9 | 560.7 | 1.48 |
| 4 45% YPC | 345.5 | 523.5 | 1.51 |
| 5 15% GPC[2] | 390.4 | 556.9 | 1.42 |
| 6 30% GPC | 362.9 | 532.9 | 1.47 |
| 7 45% GPC | 306.6 | 492.2 | 1.61 |
| SEM | 6.23 | 4.92 | 0.034 |
| Treatment contrast | | P values | |
| 1 vs 2,3,4,5,6,7 | ** | ** | ** |
| 1 vs 2,5 | NS[3] | NS | NS |
| 1 vs 3,6 | ** | NS | * |
| 1 vs 4,7 | ** | ** | ** |
| 2,5 vs 3,6 | ** | * | NS |
| 3,6 vs 4,7 | ** | ** | ** |
| 2 vs 5 | NS | NS | NS |
| 3 vs 6 | NS | ** | NS |
| 4 vs 7 | ** | ** | ** |
| 2,3,4 vs 5,6,7 | ** | ** | NS |

[1] Yellow pea chips; [2] Green pea chips; [3] Not significant.
* $P \leq 0.05$, ** $P < 0.01$.

meal at 150 g/kg had no effect ($P < 0.01$) on body weight gain, feed consumption and FCR. Weight gain was lower ($P < 0.01$) for the birds that were fed diets containing either 300 or 450 g/kg of YPC or GPC. Feed conversion ratio followed a similar trend to weight gain. Feed consumption decreased with increasing levels of YPC or GPC in the diet but the effect was significant ($P < 0.01$) only at 450 g/kg. Both products produced different effects on chick weight gain and feed consumption; however, no effects on FCR were observed (treatments 2, 3, 4 vs 5, 6, 7; see Table 35).

Early research studies on the use of peas in broiler feeding reported good performance. Brenes *et al.* (1989) reported that chicks can be grown with diets containing 800 g peas/kg diet, however, these workers did not indicate if the good performance of the birds was due to the high level of sunflower oil in the pea diet which can result in greater palatability. Investigating the effect of enzyme supplementation on the nutritional value of peas, Brenes *et al.* (1993) demonstrated that satisfactory growth performance of chicks could be obtained at 480 g peas/kg diet inclusion level. Moran *et al.* (1968) and Goatcher and McGinnis (1972) however, reported lower dietary levels of peas in their studies. At 350 g peas/kg diet, Moran *et al.* (1968) encountered a significant reduction in chick growth and feed utilization indicating that chicks could not tolerate high levels of peas in their diets as suggested by Brenes *et al.* (1989) and Brenes *et al.* (1993).

From the results of the feeding trial, it was observed that inclusion of these pea products in broiler diets at 300g/kg significantly impaired growth and feed conversion. The most pronounced effects were observed in the diets which contained 450 g/kg diet. While this poor performance could in part be explained in terms of the total sulphur

amino acid content of the diets (slightly lower than the control diet), the presence of some antinutritional factors (ANFs) in the peas which may affect nutrient utilization and performance could also contribute to it. Griffiths, (1984) and Poel *et al.* (1989) have documented the presence of protease inhibitors in peas and pea products. Lindgren, (1975) and Igbasan *et al.* (1994) showed that peas containing high level of tannins had a lower metabolizable energy content and lower amino acid availability than low tannin peas, indicating poorer nutritive value.

***Experiment 6***

Table 36 shows the performance data of broiler chicks (5-19 days) fed YPC or GPC diets supplemented with two levels of methionine to 100 and 120% of the NRC requirements (NRC, 1994). Inclusion of YPC or GPC in broiler chick diets at 300 g/kg reduced ($P < 0.01$) weight gain even at methionine levels above NRC requirement. The FCR showed a similar pattern to body weight but with the exception of methionine supplementation to 120% of the NRC requirement. Feed consumption was similar ($P \geq 0.05$) among dietary treatments. There was no difference ($P \geq 0.05$) between diets supplemented with methionine to 100 or 120% of the NRC requirements for body weight, feed consumption and FCR.

Several researchers (Moran *et al.,* 1968; Reddy *et al.,* 1979; James and Hove, 1980) have demonstrated that methionine is the first limiting amino acid in peas. Moran *et al.,* (1968) and Reddy *et al.,* (1979) reported that the addition of 0.25 and 0.2% DL-methionine, respectively, to pea diets significantly ($P < 0.05$) improved growth rate and

TABLE 36. Weight gains, feed consumption and feed conversion ratio (FCR) of broiler chicks (5-19 days) fed pea products (300 g/kg) supplemented with methionine at two levels, Experiment 6.

| Treatments | Mean weight gain, g | Mean feed consumption, g | Mean FCR, g feed/g gain |
|---|---|---|---|
| 1 Corn-soy | 438.2 | 598.2 | 1.37 |
| 2 YPC[1]-100 | 416.0 | 599.5 | 1.44 |
| 3 YPC-120 | 419.9 | 584.1 | 1.39 |
| 4 GPC[2]-100 | 413.0 | 585.4 | 1.42 |
| 5 GPC-120 | 421.8 | 596.3 | 1.41 |
| SEM | 5.56 | 3.79 | 0.022 |
| Treatment contrast | | P values | |
| 1 vs 2,3,4,5 | ** | NS[3] | * |
| 1 vs 2,4 | ** | NS | ** |
| 1 vs 3,5 | * | NS | NS |
| 2,4 vs 3,5 | NS | NS | NS |

[1] Yellow pea chips; [2] Green pea chips; [3] Not significant.
* $P \leq 0.05$, ** $P < 0.01$.

feed utilization compared with unsupplemented diets. However, these birds were still significantly smaller than those fed corn-soy diets in both studies.

The results from the current study showed significant differences ($P < 0.05$) in body weight and FCR between birds fed the control diet and diets containing pea products. However, there were differences between birds fed 300 g/kg pea products without methionine supplementation (experiment 5) and 300 g/kg pea products with methionine supplementation (experiment 6). Without methionine supplementation (experiment 2), body weight was depressed by 6% (377.9 vs 403.1 g) and 10% (362.9 vs 403.1 g) for YPC and GPC, respectively, but with methionine supplementation to 100% of the NRC requirement, body weight was only depressed by 5% (416.0 vs 438.2 g) and 6% (413.0 vs 438.2 g). A similar pattern of response to that described for body weight was noted for the FCR values. Feed consumption was also enhanced with the addition of methionine. The diets used in both experiments were similar in composition, except for methionine. Therefore, our results in this study were in good agreement with those obtained by the afforementioned authors.

Methionine supplementation to 20% above NRC requirement for broilers only produced little further (less than 5%) and non-significant responses in weight gain and FCR. These responses may not have any economic benefit for farmers when considering the cost of methionine. This indicates that the NRC recommendation of methionine requirement for broiler chicks is adequate to support the maximum growth attainable with diets containing moderate quantities of pea chips. The fact that these products at 300 g/kg inclusion level with methionine supplementation were unable to sustain broiler

performance equal to birds fed a conventional corn soybean meal diet, suggests that they should not be fed to broiler chicks in excess of 150 g/kg of the diet.

## MANUSCRIPT 5

# THE INFLUENCE OF FEEDING YELLOW-, GREEN- AND BROWN-SEEDED PEAS ON PRODUCTION PERFORMANCE OF LAYING HENS

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**ABSTRACT** Two experiments were conducted to evaluate the feeding value of yellow-, green- and brown-seeded peas as protein and energy supplements in laying hen diets. In experiment 7, all three types of peas were included in laying hen diets at 0, 200, 400 and 600 g/kg replacing wheat and soybean meal. Layers fed diets containing peas at 200 g/kg produced more ($P \leq 0.05$) eggs, had higher ($P \leq 0.05$) egg mass output and better ($P \leq 0.01$) feed conversion than those fed the wheat soybean meal control diet. With 400 g peas/kg in diets, egg production, egg mass and feed conversion were similar ($P \geq 0.05$) to the control diet. Total replacement of soybean meal with peas (600 g peas/kg in diets) reduced ($P \leq 0.01$) egg production, egg mass and feed conversion. A progressive ($P \leq 0.01$) improvement in yolk colour as the level of peas in the diet increased was observed. Shell quality decreased ($P \leq 0.05$) with increasing levels of yellow or brown peas in the diets, however, it seemed not to be affected by green peas. Daily feed intake, egg weight and mortality rates were not affected by dietary treatments. The influence of feeding diets containing yellow or brown peas supplemented with varied levels of methionine on production performance of laying hens was investigated in experiment 8. The dietary treatments consisted of a 400 g/kg yellow or brown pea diet supplemented with 3 levels of methionine to 100, 115 and 130% of the NRC requirements. Methionine supplementation to either 15 or 30% above NRC requirement for laying hens had no significant ($P \geq 0.05$) effect on all production parameters. It can be concluded that the inclusion of yellow, green or brown peas into laying hen diets up to 400 g/kg does not affect production performance. The NRC recommendation of methionine requirement for laying hens is adequate to support maximum egg production for diets containing moderate quantities of peas.

**Key words**: peas, laying hens, methionine supplementation.

## INTRODUCTION

In Western Canada, laying hen diets are based on wheat or barley with soybean meal as the major protein supplement. Soybeans are not locally produced in Western Canada but are imported. This in part contributes to high cost of egg production and it also results in total dependency on soybean meal. One approach to reducing cost and limiting dependency on soybean meal is the utilization of indigenous dietary protein sources.

Among legume crops, the production of peas (*pisum sativum L*) in Western Canada has increased dramatically within the last decade. In 1984 an estimated 73,600 ha of land was sown to peas whereas in 1994 over 640,000 ha of land was devoted to pea production (Slinkard 1994) representing a 7-8 fold increase in one decade. The nutritional advantages of peas are the relatively high contents of protein and essential amino acids, lysine and leucine (see reviews by Marquardt and Bell 1988; Savage and Deo 1989) and metabolizable energy (Igbasan *et al.*, 1994). Like other legume seeds they are deficient in methionine (Gatel 1994).

Although considerable research work has been carried out on the use of peas in diets for laying hens, published information from previous work regarding the acceptable levels of raw peas in laying hen diets are conflicting. Moran *et al.* (1968) found that 150 or 300 g/kg peas with methionine supplementation had no effect on egg production but

resulted in a significant decrease in feed conversion. In contrast, Davidson (1977 and 1980a) reported that when laying hen diets contained 170 and 370 g peas/kg, production performance was 20% and 45% lower, respectively, than the fishmeal control diet. However, with the 370 g peas/kg concentration, addition of methionine improved egg production and feed conversion but these were still lower than the control diet. In another study, Davidson *et al.* (1981) also demonstrated that egg production was reduced by 15% with 400 g/kg peas in the diets with adequate methionine supplementation. More recently, Ivusic *et al.* (1994) has shown that egg production and feed conversion were not affected when yellow peas (var. Marinda) were incorporated in diets at levels up to 590 g/kg but thinner egg shells were observed.

Studies have shown that differences exist among pea cultivars in terms of nutrient compositions and digestibilities (Carre *et al.* 1991; Gatel, 1994) and concentrations of anti-nutritional factors such as condensed tannins (Griffths, 1981) and protease inhibitors (Bacon *et al.* 1995), which may partly explain the differences in animal response. However, Davidson *et al.* (1981) did not find any significant difference between the feeding values of raw *pisum arverse* and *pisum sativum* in laying hen diets which were supplemented with adequate methionine.

Many nutritionists and feed manufacturers are concerned about these contradictory reports and they are not willing to incorporate peas into regular dietary formulation. The current study was conducted to further establish the replacement value of peas in laying hen diets. A comparison of the performance of laying hens fed yellow-, green- and brown-seeded peas will provide a basis for establishing this replacement value.

## MATERIALS AND METHODS

The yellow (*cult.* Impala), green (*cult.* Radley) and brown (*cult.* Sirius) peas used for this study were obtained from Manitoba, Canada. The chemical compositions and metabolizable energy values of these cultivars as reported by Igbasan *et al.* (1994) and Igbasan and Guenter (1996a) are given in Table 37.

### *Experiment 7*

A total of six hundred DeKalb Delta Single Comb White Leghorn pullets, 24 wk of age were housed in colony cages (40 x 30cm) at 3 birds per cage. The cages were equipped with Hart cup waterers and trough feeders. Wire grids having mesh approximately 2.5 x 2.5cm were placed on top of feeders to reduce feed spillage. The hens house was provided with programmable lighting and adequate ventilation. The lighting programme at the start of experiment was 14 h light and was increased by 15 min each wk to 16 h light.

Ten isoenergetic and isonitrogenous diets (Table 38) were formulated on the basis of ingredient analysis. Diet 1 (control diet) was a practical wheat-soybean meal laying hen diet. Diets 2 to 10 were formulated to contain 200, 400 and 600 g/kg of different types of peas which replaced wheat and soybean meal. Each diet was randomly replicated 5 times with 12 birds per replicate. The diets were presented in mash form and feed and water were provided *ad libitum* throughout the experiment. The duration of the experiment was 112 d (between 24 and 40 wk of age) and was divided into 4 periods of 28 d each.

TABLE 37. Chemical compositions (g/kg DM basis) of the pea cultivars[1].

| Parameters | Peas | | |
|---|---|---|---|
| | Impala (Yellow) | Radley (Green) | Sirius (Brown) |
| Crude protein (N x 6.25) | 245.2 | 232.2 | 231.9 |
| Dietary Fibre | 192.3 | 192.5 | 223.1 |
| Starch | 400.8 | 425.1 | 434.0 |
| Ash | 30.6 | 25.8 | 29.5 |
| Fat (ether extract) | 14.3 | 20.1 | 12.4 |
| Calcium | 1.3 | 0.7 | 0.8 |
| Phosphorus | 4.3 | 4.4 | 4.4 |
| Tannin | < 1.0 | < 1.0 | 41.0 |
| Cystine, g per 16g N | 1.5 | 1.6 | 1.7 |
| Lysine, g per 16g N | 7.2 | 7.5 | 7.4 |
| Methionine, g per 16g N | 1.2 | 0.9 | 0.9 |
| Metabolizable energy, TMEn, MJ/kg | 11.7 | 12.6 | 11.0 |

[1] Adapted from Igbasan *et al.* (1994) and Igbasan and Guenter (1996a)

TABLE 38. Composition of experimental diets (g/kg), Experiment 7.

| Ingredients | Control | Impala (Yellow) | | | Radley (Green) | | | Sirius (Brown) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| Peas | - | 200.0 | 400.0 | 600.0 | 200.0 | 400.0 | 600.0 | 200.0 | 400.0 | 600.0 |
| Soybean | 140.0 | 88.0 | 43.0 | - | 96.5 | 55.6 | - | 102.1 | 65.5 | - |
| Wheat | 617.9 | 465.0 | 304.4 | 138.9 | 462.3 | 304.0 | 161.3 | 443.2 | 267.8 | 234.0 |
| Barley | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | - |
| Limestone | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 |
| Biophos | 10.0 | 10.0 | 9.0 | 9.0 | 10.0 | 9.0 | 9.0 | 10.0 | 9.0 | 9.0 |
| DL-Met. | 1.0 | 1.0 | 1.1 | 1.1 | 1.2 | 1.4 | 1.7 | 1.2 | 1.3 | 1.5 |
| Lysine | 1.1 | - | - | - | - | - | - | - | - | - |
| Vitamin mix[1] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral mix[2] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vegetable oil | 28.0 | 34.0 | 40.5 | 49.0 | 28.0 | 28.0 | 26.0 | 41.5 | 54.4 | 53.5 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| Calculated analysis, g/kg | | | | | | | | | | |
| TMEn, MJ/kg | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.7 |
| Protein | 183.4 | 182.0 | 183.7 | 185.4 | 183.0 | 184.3 | 181.7 | 182.3 | 182.6 | 180.5 |
| Calcium | 35.5 | 35.6 | 35.5 | 35.6 | 35.5 | 35.3 | 35.2 | 35.5 | 35.3 | 35.3 |
| Avail. P. | 3.5 | 3.5 | 3.5 | 3.5 | 3.6 | 3.5 | 3.6 | 3.6 | 3.5 | 3.5 |
| Met. | 3.8 | 3.8 | 3.9 | 3.9 | 3.8 | 3.8 | 3.8 | 3.9 | 3.8 | 3.8 |
| Met. + Cys. | 6.7 | 6.5 | 6.4 | 6.3 | 6.6 | 6.5 | 6.5 | 6.6 | 6.4 | 6.2 |
| Lysine | 8.1 | 8.1 | 9.1 | 10.1 | 8.4 | 9.7 | 10.7 | 8.1 | 9.0 | 9.2 |

[1] Amount supplied per kg of diet: Vitamin A, 8250 IU; Cholecalciferol, 1000 IU; Vitamin E, 5.46 IU; Vitamin $B_{12}$, 0.112 mg; Riboflavin, 2.2 mg; Niacin, 6.6 mg; Ca-pantothenate, 4.4 mg; Choline chloride, 110 mg; DL-methionine, 500 mg.

[2] Amount supplied per kg of diet: Manganese 110, mg; Zinc, 55 mg; Iodized salt, 4780 mg.

Egg production was recorded daily and the data were pooled into four 28-day periods. Feed consumption was determined at the end of each 28 d period. Individual body weights were obtained at the start and end of the experiment. Mortality was recorded as it occurred.

On the last 3 consecutive days of each of the four 28-day periods, eggs gathered were used for exterior and interior egg quality determinations. Average egg weight was recorded for each replicate and egg specific gravity was determined using the procedure outlined by Hamilton (1982). Albumen height was measured using an electronic albumen height guage[1], yolk colour was determined by the Roche yolk colour fan[2] (15, dark orange; 1, light pale) and shell thickness measurement was taken using the Ames micrometer[3]. Feed conversion and egg mass (rate of lay x egg weights) were calculated from the data.

***Experiment 8***

In experiment 8, the influence of varied levels of methionine supplementation on the productive performance of laying hens fed a moderate level (400 g/kg diet) of yellow and brown peas was investigated. Analytical data on amino acid content of peas have shown that methionine is the first limiting amino acid for poultry (Reddy *et al.* 1979;

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[1]Qeensboro intruments, 645 Brierwood, Ottawa, Canada.

[2]Hoffman-La Roche Ltd., Basel, Switzerland.

[3]B.C. Ames, Co. Waltham, Mass.

NRC, 1994) and most often pea-based diets are formulated on the basis of total methionine content. Our previous work (Igbasan *et al.* 1994) showed that the availabilities of methionine as well as cystine were lower than the availabilities of other amino acids which would further aggravate the deficiency of these amino acids. Supplemental methionine in excess of NRC recommendation may be beneficial. The yellow and brown peas were used for this experiment because the yellow is tannin-free (Table 37) and widely grown in Western Canada while the brown contained appreciable quantities of tannin.

Two hundred and forty Shaver Single Comb White Leghorn (SCWL) pullets housed in colony cages (40 x 25 cm) at 2 birds per cage were randomly allotted at 25 wk of age to 6 dietary treatments with 10 replicates of 4 birds each per treatment. The dietary treatments consisted of a 400 g/kg Impala (yellow) or Sirius (brown) pea diet (Table 39) supplemented with 3 levels of methionine to 100, 115 and 130% of NRC requirements (NRC, 1994). With the exception of methionine, all other nutrients were formulated to meet NRC requirements (NRC, 1994). The duration of the experiment was 84 d (between 25 and 37 wk of age) and was divided into 3 periods of 28 d each. Feed and water were provided *ad libitum.*

Hen-day egg production, feed consumption and mortality data were recorded throughout the experimental period. Mean egg weight was determined for each replicate by measuring the weight of eggs laid on two consecutive days in every two weeks. Egg specific gravity, Albumen height, yolk colour and shell thickness determinations were made, as described for experiment 7, on all eggs gathered on the last 3 consecutive days

TABLE 39. Composition of experimental diets (g/kg), Experiment 8.

| Ingredients | Impala (Yellow) | | | Sirius (Brown) | | |
|---|---|---|---|---|---|---|
| | Methionine level, % NRC | | | | | |
| | 100 | 115 | 130 | 100 | 115 | 130 |
| Peas | 400.0 | 400.0 | 400.0 | 400.0 | 400.0 | 400.0 |
| Soybean | 43.0 | 43.0 | 43.0 | 65.5 | 65.5 | 65.5 |
| Wheat | 304.4 | 303.8 | 303.3 | 267.8 | 267.2 | 266.7 |
| Barley | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| Limestone | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 |
| Biophos | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| DL-Met. | 1.1 | 1.7 | 2.2 | 1.3 | 1.9 | 2.4 |
| Vitamin mix[1] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral mix[2] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vegetable oil | 40.5 | 40.5 | 40.5 | 54.4 | 54.4 | 54.4 |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| | | | | | | |
| Calculated analysis, g/kg | | | | | | |
| TMEn, MJ/kg | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 | 11.8 |
| Protein | 183.7 | 183.9 | 184.1 | 182.6 | 182.8 | 183.0 |
| Calcium | 35.5 | 35.5 | 35.5 | 35.3 | 35.3 | 35.3 |
| Avail. P. | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Met. | 3.9 | 4.4 | 4.9 | 3.8 | 4.4 | 4.9 |
| Met. + Cys. | 6.4 | 7.0 | 7.5 | 6.4 | 6.9 | 7.5 |
| Lysine | 9.1 | 9.1 | 9.1 | 9.0 | 9.0 | 9.0 |

[1, 2] Same as Table 38.

of each three 28-day periods. Hens were weighed at the beginning and at the end of the experiment.

### *Statistical Analysis*

Data were analyzed using the General Linear Model procedures of the SAS® Institute, Inc. (1986). When analysis of variance indicated a significant treatment effect, linear contrasts (experiment 1) and Duncan multiple range test (Duncan 1955) (experiment 2) were used to compare treatment means.

## RESULTS AND DISCUSSION

### *Experiment 7*

The productive performance of laying hens fed diets varying in yellow-, green- and brown-seeded peas is shown in Table 40. Egg quality parameters as affected by dietary treatments are summarized in Table 41. Layers fed diets containing all three types of peas at 200 g/kg diet produced more ($P \leq 0.05$) eggs, had higher ($P \leq 0.05$) egg mass output and better ($P \leq 0.01$) feed conversion than those fed the wheat-soybean meal control diet. This could probably be due to the fact that at this level of inclusion a better balanced nutrient profile was achieved, for instance threonine, arginine as well as linoleic acid were slightly higher in these diets than in the wheat-soybean meal control diet. With 400 g peas/kg in diets, egg production, egg mass and feed conversion were similar ($P \geq 0.05$) to the control diet. When the concentration of peas in the diets was increased to 600 g/kg, egg production was lower ($P \leq 0.01$) than the control diet by about 4.6, 5.5 and

TABLE 40. The influence of feeding varying levels of yellow, green and brown peas on production performance of laying hens (24-40 weeks of age), Experiment 7.

| Dietary inclusion of peas, g/kg | Rate of lay, % | Daily feed intake, g/bird | Feed conv., kg/doz. | Egg mass output[1], g/day | Body weight, kg | Body weight change, kg | Mortality rate, % |
|---|---|---|---|---|---|---|---|
| 1 0 | 89.8 | 110.0 | 1.48 | 53.6 | 1.69 | 0.11 | 3.3 |
| 2 MP[2]-200 | 91.1 | 108.8 | 1.44 | 53.3 | 1.71 | 0.10 | 1.7 |
| 3 MP-400 | 90.1 | 110.5 | 1.47 | 53.2 | 1.70 | 0.09 | 3.3 |
| 4 MP-600 | 85.7 | 110.1 | 1.54 | 49.5 | 1.69 | 0.07 | 0 |
| 5 RD[3]-200 | 91.5 | 109.0 | 1.43 | 54.2 | 1.73 | 0.09 | 1.7 |
| 6 RD-400 | 89.1 | 110.3 | 1.48 | 52.8 | 1.70 | 0.08 | 1.7 |
| 7 RD-600 | 84.9 | 109.2 | 1.54 | 49.8 | 1.68 | 0.07 | 3.3 |
| 8 SR[4]-200 | 92.6 | 109.2 | 1.42 | 55.5 | 1.72 | 0.09 | 1.7 |
| 9 SR-400 | 89.9 | 109.3 | 1.47 | 52.3 | 1.72 | 0.08 | 1.7 |
| 10 SR-600 | 85.4 | 111.8 | 1.57 | 50.2 | 1.67 | 0.07 | 3.3 |
| SEM | 0.79 | 0.88 | 0.014 | 0.84 | ND[5] | 0.012 | ND |
| Treatment contrast | P Values | | | | | | |
| I vs all others | NS | NS[6] | * | * | ND | * | ND |
| 1 vs 2,5,8 | * | NS | ** | * | ND | NS | ND |
| 1 vs 3,6,9 | NS | NS | NS | NS | ND | NS | ND |
| 1 vs 4,7,10 | ** | NS | ** | * | ND | ** | ND |
| 2,5,8 vs 3,6,9 | * | NS | ** | * | ND | NS | ND |
| 2,5,8 vs 4,7,10 | ** | NS | ** | ** | ND | * | ND |
| 3,6,9 vs 4,7,10 | * | NS | ** | * | ND | NS | ND |
| 2 vs 5,8 | NS | NS | NS | NS | ND | NS | ND |
| 3 vs 6,9 | NS | NS | NS | NS | ND | NS | ND |
| 4 vs 7,10 | NS | NS | NS | NS | ND | NS | ND |

* Effect significant at $P \leq 0.05$; ** Effect significant at $P \leq 0.01$.
[1] Rate of lay x average egg weight.
[2] Impala; [3] Radley; [4] Sirius
[5] Not determined.
[6] Not significant.

TABLE 41. The influence of feeding varying levels of yellow, green and brown peas on egg quality parameters, Experiment 7.

| Dietary inclusion of peas, g/kg | Egg quality parameters | | | | |
|---|---|---|---|---|---|
| | Egg weight, g | Yolk colour score[1] | Albumen height, mm | Shell thickness, mm x$10^{-2}$ | Specific gravity[2] |
| 1 0 | 59.7 | 2.8 | 8.5 | 35.1 | 1.0839 |
| 2 MP-200 | 58.5 | 3.8 | 8.6 | 34.0 | 1.0829 |
| 3 MP-400 | 59.0 | 4.4 | 8.8 | 33.8 | 1.0828 |
| 4 MP-600 | 57.7 | 4.9 | 8.6 | 33.5 | 1.0828 |
| 5 RD-200 | 59.2 | 4.1 | 8.6 | 34.5 | 1.0834 |
| 6 RD-400 | 59.3 | 4.8 | 8.7 | 34.2 | 1.0833 |
| 7 RD-600 | 58.6 | 5.7 | 8.5 | 35.3 | 1.0845 |
| 8 SR-200 | 59.9 | 4.0 | 8.8 | 35.2 | 1.0841 |
| 9 SR-400 | 58.1 | 4.7 | 8.6 | 33.8 | 1.0829 |
| 10 SR-600 | 58.7 | 5.3 | 8.6 | 33.7 | 1.0819 |
| SEM | 0.67 | 0.11 | 0.10 | 0.22 | 0.00033 |
| Treatment contrast | P Values | | | | |
| 1 vs all others | NS[3] | ** | NS | ** | * |
| 1 vs 2,5,8 | NS | ** | NS | NS | * |
| 1 vs 3,6,9 | NS | ** | NS | * | * |
| 1 vs 4,7,10 | NS | ** | NS | ** | ** |
| 2,5,8 vs 3,6,9 | NS | ** | NS | NS | NS |
| 2,5,8 vs 4,7,10 | NS | ** | NS | * | * |
| 3,6,9 vs 4,7,10 | NS | ** | NS | * | * |
| 2 vs 5,8 | NS | NS | NS | NS | * |
| 3 vs 6,9 | NS | * | NS | * | * |
| 4 vs 7,10 | NS | ** | NS | ** | ** |

* Effect significant at $P \leq 0.05$.
** Effect significant at $P \leq 0.01$.
[1] Egg yolk colour based on Roche colour fan: 15, dark orange; 1, light pale yellow.
[2] Determined by dipping eggs into tanks of salt water with specific gravity ranging from 1.075 to 1.100, increasing in increments of 0.005.
[3] Not significant.

4.9% for yellow, green and brown peas respectively. Egg mass and feed conversion followed the same trend.

Figure 5 shows that egg production consistently increased over the course of the experiment for layers fed diets containing 200 g peas/kg diet. However, the effects of incorporating either 400 or 600 g peas/kg diet on egg production started to manifest at about 12 weeks after the experiment began. Daily feed intake was similar ($P \geq 0.05$) for all treatment groups. Decreased ($P \leq 0.01$) body weight gain was observed among pullets fed diets containing 600 g/kg. Mortality rates did not appear to be related to treatments applied.

Inclusion of peas up to 600 g/kg diet did not affect egg weight nor albumen height. However, a progressive ($P \leq 0.01$) improvement in yolk colour as the level of peas in the diet increased was observed. The response of egg shell quality (shell thickness and specific gravity) was not the same for the three pea cultivars. Shell quality decreased ($P \leq 0.05$) with increasing level of yellow or brown peas in the diets, however, it seemed not to be affected by green peas.

The present results indicate that yellow, green and brown peas constitute adequate sources of supplemental protein and energy for regular use in laying hen diets. Generally, optimum production performance was maintained with the inclusion of peas up to 400 g/kg diet with adequate supplementation of methionine. Only at levels in excess of 400 g/kg of the total diet was there an indication of a detrimental effects on production characteristics.

These data in part are in agreement with those of Moran *et al.* (1968), Anderson

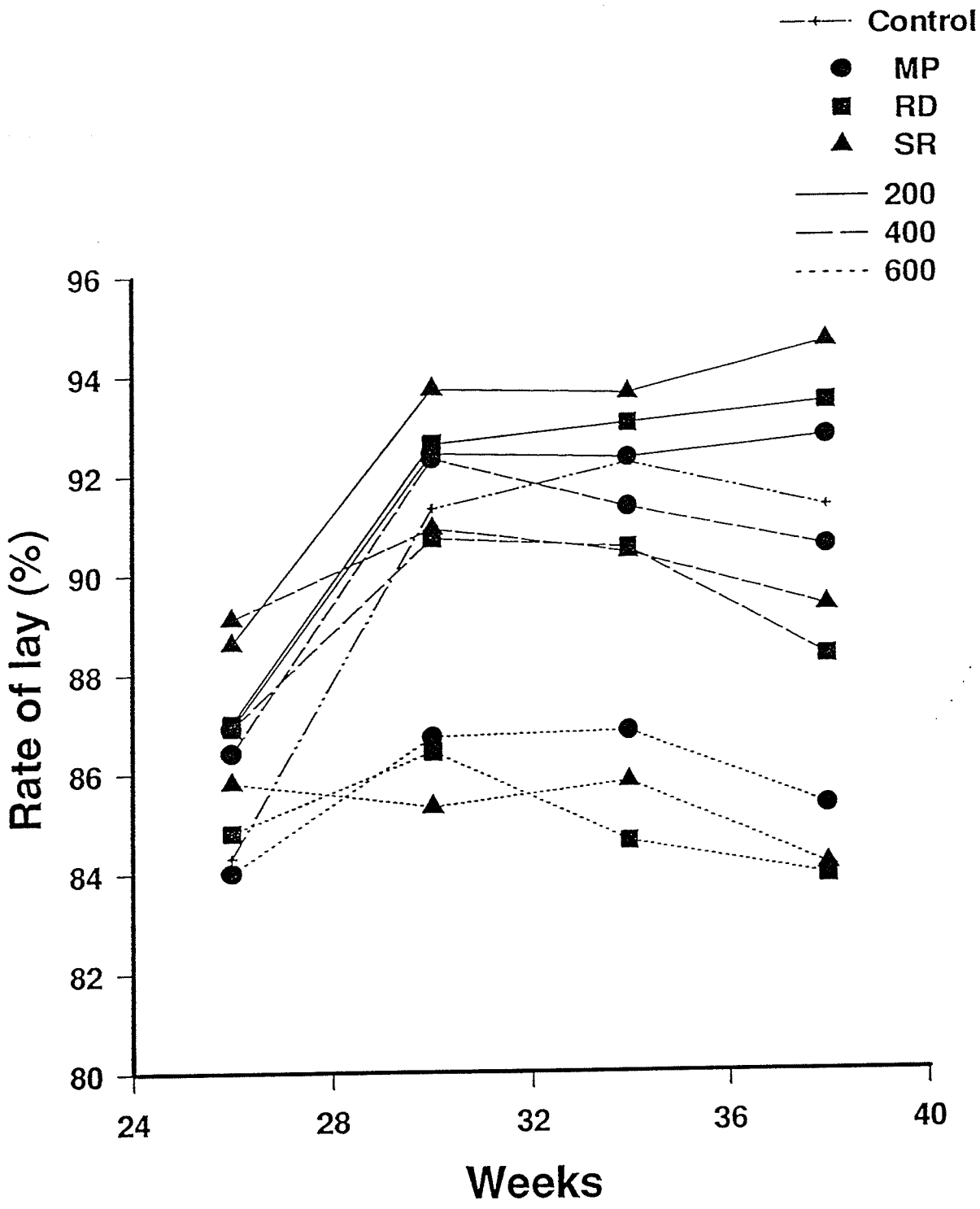


Fig 5 Daily egg production during the experimental period as affected by diets.

(1979), Davidson *et al.* (1981), Castanon and Perez-Lanzac (1990) and Ivusic *et al.* (1994). Moran *et al.* (1968) found no effect on egg production but a depression in feed conversion when diets containing peas at level of 300 g/kg were fed to laying hens. In a short term (8 wk) study, Castanon and Perez-Lanzac (1990) included up to 500 g/kg peas into laying hen diets and observed no negative effect on egg production. Ivusic *et al.* (1994) also reported no effect on laying performance when peas constituted up to 590 g/kg of their diets. Our data showed that inclusion of peas into laying hen diets at 600 g/kg produced depression in egg production and feed conversion. These inconsistencies may in part be attributed to differences in experimental methodology and as well to differences in the quality of peas used in different studies. Recently, we (Igbasan *et al.*, 1994) evaluated twelve newly registered pea cultivars in Western Canada for nutritional quality and observed that there were considerable variations in protein and metabolizable energy (TMEn) contents among these cultivars. Griffiths (1981 and 1984) also documented the presence and the variation of protease inhibitors and condensed tannins in peas, which could affect nutrient utilization and performance of birds. However, we did not observe major differences in production responses among the cultivars tested in the present study. Although the cultivars showed some degree of variation in their chemical compositions (with respect to methionine and tannin contents) and metabolizable energy values (Table 37) but by supplementing diets with adequate synthetic methionine and vegetable oil these variations were eliminated. It seemed that the tannin content in the brown cultivar is not high enough to influence production performance. A similar observation has been made by Davidson *et al.* (1981) who fed diets containing *Pisum*

*arverse* and *Pisum sativum* supplemented with adequate methionine to laying hens and found no significant difference in production performance. The authors did not indicate if the chemical composition of the peas were similar. These results indicate that if layer diets are properly balanced for energy and amino acids especially methionine, equivalent performance can be achieved between cultivars of peas provided they contain levels of toxic substances which the animal can cope with physiologically. However, this may require regular analysis to determine the contents of those nutrients which are of concern in diet formulation.

It may be noted (Fig. 5) that the detrimental effects of feeding high concentrations of peas on egg production became apparent after 12 weeks of the experiment. This information was consistent with that reported by Davidson *et al.* (1981), where egg production declined when peas were fed at high concentrations for long periods. It is also possible that a lack of adverse effect on performance observed by Castanon and Perez-Lanzac (1990) may be due to the duration of their experiment. The positive influence of peas on yolk colour observed in the present study may be related to the quantity of xanthophylls in peas, but at present there is no information to support the observation. Also there was no apparent explanation for the differences in response of shell quality to diets containing yellow, green and brown peas.

***Experiment 8***

The influence of feeding diets containing yellow or brown peas supplemented with varied levels of methionine on production performance of laying hens is presented in

Table 42. Methionine supplementation to either 15 or 30% above NRC requirement for laying hens had no significant ($P \geq 0.05$) effect on all production parameters evaluated in this study. However, slight improvement in egg production, egg mass output and body weight gain was observed in laying hens fed the diet containing brown peas supplemented with methionine to 15% above NRC requirement. The small response may be related to the tannin content of this particular cultivar. But results obtained from this experiment and from experiment 7 suggest that although the brown peas used here contained tannin, the tannin content is not high enough to influence production performance when brown peas are included in laying hen diets up to 400 g/kg. Investigating the effects of sorghum tannin on laying hen performance, Maier *et al.* (1978) has shown that dietary tannin concentrations of 9.4 g/kg diet had no effect on egg production or feed conversion. The highest concentration of tannin in our experimental diets was 2.7 g/kg (calculated). The significant ($P \leq 0.05$) difference observed in yolk colour was not related to methionine supplementation but to the type of peas.

The results from this experiment do not agree with those of Davidson (1980a) and Davidson *et al.* (1981) who reported that methionine supplementation significantly improved egg production by about 20%. In the experiments conducted by Davidson (1980) and Davidson *et al.* (1981) the control diets which contained raw peas were critically low in methionine (1.9 g/kg) so supplementation with methionine produced significant improvement in egg production, whereas in our study care was taken that methionine was added up to the laying hen's requirement so further addition of methionine did not have effect on performance. This low level of methionine in their

TABLE 42. Production performance of laying hens (25-37 weeks of age) fed yellow or brown peas (400g/kg) supplemented with varied levels of methionine, Experiment 8.

| Dietary treatments | Rate of lay, % | Daily feed intake, g/kg | Feed conv., kg/doz. | Egg weight, g | Egg mass output[1], g/day | Yolk colour score[2] | Shell thickness, mmx$10^{-2}$ | Specific gravity[3] | Albumen height, mm | Body weight change, kg | Mortality rate, % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MP-100[4] | 92.9 | 105.8 | 1.40 | 57.4 | 53.3 | 4.8a | 35.7 | 1.0838 | 9.1 | 0.05 | 2.5 |
| MP-115 | 90.5 | 106.2 | 1.41 | 57.9 | 52.4 | 4.7a | 35.7 | 1.0843 | 9.2 | 0.05 | - |
| MP-130 | 91.1 | 107.4 | 1.46 | 57.5 | 52.4 | 4.8a | 36.0 | 1.0848 | 9.1 | 0.06 | - |
| SR-100 | 91.9 | 105.9 | 1.43 | 57.6 | 52.9 | 5.2b | 36.1 | 1.0841 | 9.2 | 0.06 | - |
| SR-115 | 93.6 | 105.9 | 1.39 | 57.9 | 54.2 | 5.2b | 36.1 | 1.0850 | 9.0 | 0.08 | 2.5 |
| SR-130 | 92.9 | 106.2 | 1.43 | 57.7 | 53.5 | 5.1b | 36.4 | 1.0851 | 9.0 | 0.07 | 2.5 |
| SEM | 1.40 | 0.76 | 0.028 | 0.45 | 0.85 | 0.05 | 0.34 | 0.00082 | 0.13 | 0.013 | ND[5] |

[1] Same as Table 40; [2, 3] Same as Table 41.
[4] Level of methionine in diet (% of NRC, 1994).
[5] Not determined.
a, b Means within column followed by different letters differ significantly ($P \leq 0.05$).

control diet coupled with poor availability of methionine from peas may explain the differences in results.

From experiment 7 it can be concluded that inclusion of yellow, green or brown peas into laying hen diets up to 400 g/kg does not affect production performance when the diets are carefully balanced to meet optimum nutrient requirements for laying hens. Results from experiment 8 demonstrate that the NRC recommendation of methionine requirement for laying hens is adequate to support maximum egg production for diets containing moderate quantities of peas.

# CHAPTER FIVE

## IMPROVEMENTS OF THE NUTRITIVE VALUE OF PEAS

There are two manuscripts in this chapter, manuscript 6 & 7. The effects of micronization and dehulling on the nutritive value of peas for broiler chickens were discussed in manuscript 6 while in manuscript 7, the effects of micronization, dehulling and enzyme supplementation on the nutritive value of peas for laying hens were addressed.

## MANUSCRIPT 6

# THE ENHANCEMENT OF THE NUTRITIVE VALUE OF PEAS FOR BROILER CHICKENS: AN EVALUATION OF MICRONIZATION AND DEHULLING PROCESSES

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**ABSTRACT** The effects of dehulling and micronization (infrared heating) on the nutritive value of three cultivars (Impala, Radley, and Sirius) of peas (*Pisum sativum*) varying in coat colour for broiler chickens were studied. Dehulling increased ($P \leq 0.05$) protein and starch contents and decreased ($P \leq 0.05$) fibre content of peas. Also, the concentrations of most amino acids slightly increased ($P \geq 0.05$). Micronization slightly changed ($P \geq 0.05$) the chemical composition of peas. Moisture, protein, fibre and phosphorus contents marginally decreased. The concentrations of aspartic acid, glutamic acid, isoleucine, lysine, cystine, threonine, and tyrosine decreased ($P \leq 0.05$) whereas that of methionine slightly increased ($P \geq 0.05$). Lysine concentration was decreased by 2.7%. The $TME_n$ and average true AA availability values determined with adult cockerels were improved ($P \leq 0.05$) by micronization, but the availabilities of lysine were decreased by 11.3, 4.4, and 2.0% for Impala, Radley and Sirius, respectively. The decrease was significant ($P \leq 0.05$) for Impala and Radley but not significant for Sirius peas. Application of micronization resulted in a significant ($P \leq 0.05$) improvement in $AME_n$, apparent protein digestibility (APD) and starch digestibility. The increments ranged from 19.4 to 31.3, 8.5 to 33.6, and 12.2 to 22.3% for $AME_n$, APD, and starch digestibility, respectively. In contrast, dehulling had little or no effect on $AME_n$ (3.0 and 4.9%), APD (- 1.8 and - 4.2%) and starch digestibility (2.0 and 1.3%) values of Impala (yellow-seeded) and Radley (green-seeded) peas but significantly ($P \leq 0.05$) improved the $AME_n$ (24.1%), APD (26.8%), and starch digestibility (12.1%) values of Sirius (brown-seeded) peas. Chick performance was evaluated in a 2-wk trial. Birds fed micronized peas grew faster and had better feed conversion ($P \leq 0.05$) than birds fed untreated peas and the wheat-soybean control diet. Addition of lysine to the diet containing micronized peas further improved ($P \geq 0.05$) weight gains and feed conversion of broiler chicks.

Dehulling only improved ($P \leq 0.05$) the performance of birds fed Sirius peas.

**Key words:** peas, *pisum sativum*, micronization, dehulling, broiler

## INTRODUCTION

Like other grain legumes, peas (*Pisum sativum* L) can provide a considerable proportion of dietary protein and energy for poultry; however, the inclusion of high amounts (above 20%) of peas in diets of broiler chickens has been reported to have a negative effect on growth rate and feed utilization ( Moran *et al.*, 1968; Igbasan and Guenter, 1996a). This negative effect has generally been attributed to the presence of various antinutritive or toxic substances in peas. Heat-labile protease inhibitors (Griffiths, 1984), tannins (Griffiths, 1981; Igbasan *et al.*, 1994), and lectins (Bender, 1983) are the antinutritional factors most often implicated in the poor performance of chickens fed diets containing peas.

Compared with cereal grains, pea seeds are slightly lower in starch, which is the greatest single dietary source of energy; however, energy value in peas is lower than in cereal grains. Pea starch is considerably less digestible by chickens than starch in any of the cereal grains (Longstaff and McNab, 1987). Although factors such as particle size and nature of starch granules (Moran, 1982), antinutritional factors like amylase inhibitors, lectins, tannins and phytates (Thorne *et al.*, 1983; Dreher *et al.*, 1984) may contribute to poor digestibility of pea starch in poultry, Longstaff and McNab (1987) suggested that accessibility of starch granules to enzymic attack, which is dependent on endosperm cell

wall thickness and structure, seems to play a greater role.

Processing methods that eliminate or inactivate toxic substances from peas and alter starch structure to improve accessibility of starch granules to enzyme degradation offer promise of improving nutrient utilization and allowing maximum use of peas in poultry diets.

The overall performance of chickens fed peas has been shown to be influenced by heat-treatment of the peas (Longstaff and McNab, 1987; Conan and Carre 1989; Brenes *et al.*, 1993). Those studies demonstrated that autoclaving improves protein and starch digestibilities and apparent metabolizable energy values of peas. However, the potential damage of protein is high because of treatment time and also it is an impractical process for large-scale commercial production required by the feed industry.

Heat processing, such as micronization, which ensures continuous flow of grains and shorter treatment time to minimize protein damage or treatment based on the separation of whole grains into fractions with high and low levels of antinutritional factors, such as dehulling, may be more beneficial than autoclaving. Micronization is the name given to a process in which industrial propane is burned over ceramic tile or nichrome wire elements to produce infrared radiation, which, when absorbed by grains, is reported to cause rapid internal heating and subsequent starch gelatinization (McNab and Wilson, 1974). The process has been reported to improve the nutritive quality of cereal grains for growing pigs (Lawrence, 1973; Savage *et al.*, 1980) and chickens (Douglas *et al.*, 1991) and faba bean (*Vicia faba* L.) (McNab and Wilson, 1974) for growing chickens. There is no documented report to show whether micronization would

have any beneficial effect on the nutritive value of peas.

The present study was undertaken 1) to determine the effects of micronization and dehulling on the chemical composition and nutrient availabilities of three cultivars of peas differing in seed coat colour; 2) to examine the growth performance of broiler chickens fed micronized and dehulled peas; 3) to determine whether lysine supplementation of micronized peas would have any effect on the chick performance.

## MATERIALS AND METHODS

### *Plant Materials*

Three cultivars of peas, Impala, Radley, and Sirius, representing yellow, green, and brown seed coat colour respectively, were obtained from Manitoba, Canada. The chemical compositions of these cultivars have been reported (Igbasan and Guenter, 1996a).

### *Pea Processing*

Prior to micronizing, the peas, having an average moisture content of 12.3% were reconstituted to about 18% moisture. Reconstitution or tempering was carried out by adding tap water to a 250-kg sample of each cultivar in a medium-sized vertical silo. The tempering took 18 h. The tempered peas were micronized (infrared heated) at a temperature between 110 and 115 C for 55 s in a micronizer[1] located at InfraReady

Products Limited, Saskatoon, Saskatchewan, S7K 5Y8, Canada. Micronizing was immediately followed by a roll flaking process in a flaking mill located just posterior to the micronizer.

The dehulling process involved cracking of the hulls with a roller mill followed by air classification to separate the hulls from the cotyledons. Apart from grinding, which was also performed on processed samples, there was no further processing carried out on the peas samples used as control.

***Chemical Analyses***

All pea samples were analyzed in duplicate for DM, CP (N x 6.25), NDF, starch, ash, fat (ether extract), calcium, phosphorus, and AAs. Chemical analyses were also performed on the feed and excreta samples obtained from the digestibility trials.

The DM, CP, ash, and fat were analyzed by the standard methods of the Association of Official Analytical Chemists (AOAC, 1984). Calcium and total phosphorus were determined by the procedures of the AOAC (1990) and Analytical Methods for Atomic Absorption Spectrophotometry (Perkin-Elmer, 1973). The NDF content was measured by the procedure outlined by Van Soest and Wine (1967) and modified by Robertson and Van Soest (1977) with the addition of alpha amylase enzyme (Termamyl)[2].

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[1]Micronizing Co., UK, Framlingham, Suffolk, England.

[2]Novo Nordisk A/S, Bagsvaerd, Denmark.

Starch in peas, feed, and excreta samples was taken as the difference between the total glucose and nonstarch polysaccharide (NSP) glucose. Total glucose was measured using a modified NSP determination procedure described by Englyst and Cummings (1984) with minor modifications (Slominski and Campbell, 1990). The modification included the elimination of the enzyme hydrolysis step in the procedure after the sample had been gelatinized-solubilized in a boiling water bath for 0.5 h in 0.1 *M* sodium acetate buffer. The total glucose and the NSP glucose were quantified by using gas-liquid chromatograghy.

Samples were analyzed for AA content with a LKB 4151 Alpha Plus AA Analyzer[3]. One hundred milligrams of each sample were prepared by acid hydrolyis using the method of AOAC (1984) as modified by Mills *et al.* (1989). Acid hydrolysis involved digestion in 4 *ml* of 6 *N* Hcl for 24 h at 110 C. Methionine and cystine were detremined by the method of Hirs (1967). The gross energy content of peas, feed, and excreta samples was measured using a Parr adiabatic oxygen bomb calorimeter[4].

***Digestibility Trials***

Digestibility trials were performed with adult cockerels and broiler chicks. The precision-feeding technique described by Sibbald (1986) with minor modifications (Zhang *et al.*, 1994) was used to determine the $TME_n$ and TAAA values of untreated and

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[3]LKB Biochrom Ltd., Cambridge, UK.

[4]Parr Instrument Co., Moline, IL.

micronized peas in adult cockerels.

Briefly, following a 28-h period without feed, each pea sample was precision-fed (30 g per bird) to a group of 10 birds housed in individual metabolism cages (62.2 x 34.3 x 43.3 cm) in an environmentally controlled room. After 48 h, all excreta from each bird was collected. The excreta samples were frozen, freeze-dried, and ground for chemical analyses. The $TME_n$ values were calculated according to the method of Sibbald (1986) and the TAAA were calculated as described by Sibbald (1979). Endogenous energy, nitrogen, and amino acid values used in the calculations were obtained from pooled data for 30 unfed birds treated as described for the precision-fed birds. The $TME_n$ and TAAA values for each pea sample were determined in duplicate and each duplicate value represent a pooled determination from 10 birds.

A 9-d feeding trial was also conducted with broiler chicks to determine the $AME_n$, apparent protein digestibility (APD) and starch digestibility of untreated, micronized and dehulled peas. Two hundred and forty day-old Arbor Acres male broiler chicks were obtained from a commercial hatchery and housed in electrically heated Petersime battery brooders[5]. The brooders were housed in an environmentally controlled room and the temperature in the brooders was regulated to be 35 C for week 1 and 32 C for week 2. The room temperature was maintained at 25 C.

The trial was conducted using a modified total excreta collection procedure as described by Mollah *et al.* (1983). A corn-soybean diet was formulated (Table 43) as a

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[5]Petersime Incubator Co., Gettysburg, OH 45328.

TABLE 43. Composition of basal diet, Experiment 9.

| Ingredients | Composition, g/kg |
|---|---|
| Soybean meal | 351.0 |
| Corn | 600.1 |
| Limestone | 19.0 |
| Biophos | 13.5 |
| DL-methionine | 1.4 |
| Total[1] | 985.0 |
| | |
| Chemical analysis, g/kg | |
| Dry matter | 874.8 |
| AME, Mcal/kg | 3.11 |
| Crude protein | 227.7 |
| Lysine | 11.6 |
| Methionine | 5.1 |
| Met + Cys | 9.5 |
| Calcium | 10.6 |
| Avail. Phosphorus | 5.1 |

[1] To avoid vitamin and mineral imbalances, these two nutrient mixes were added during final mixing, representing 15.0 g/kg of diet composition. The final mixing proportions per kilogram of diet were: Basal diet - 485.0, peas - 500.0, vitamin mix - 10.0, mineral mix - 5.0 g.
For the vitamix mix, the amount supplied per kilogram of diet were: Vitamin A, 8250 IU; Cholecalciferol, 991 IU; Vitamin E, 11.0 IU; Vitamin $B_{12}$, 11.5 $\mu$g; Vitamin K, 1.1 mg; Riboflavin, 5.5 mg; Ca-pantothenate, 11.0 mg; Niacin, 53.0 mg; Choline chloride, 1020 mg; Folic acid, 0.75 mg; Biotin, 0.25 mg; Delaquin (ethoxyquin - anti-oxidant), 125.0 mg; Methionine, 500 mg.
For the mineral mix, the amount supplied per kilogram of diet were: Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg, I, 0.8 mg.

reference (basal) diet. The test materials (untreated and treated peas) were substituted for 500 g/kg of the basal diet. However, to avoid vitamin and mineral deficiencies, components of the diet containing these nutrients were left intact and were added during final mixing. The mixing proportions for all diets were as follows: basal diet, 485 g; peas, 500 g, and vitamin and mineral premixes, 15 g.

Ten diets were prepared. The diets were as follows: corn-soybean basal diet (CS); CS +untreated Impala (MP) peas (50:50); CS + dehulled MP (50:50); CS + micronized MP (50:50); CS + untreated Radley (RD) peas (50:50); CS + dehulled RD (50:50); CS + micronized RD (50:50); CS +untreated Sirius (SR) peas (50:50); CS + dehulled SR (50:50); and CS + micronized SR (50:50). Each dietary treatment was replicated with six pens of four birds per pen.

Birds were maintained on a standard diet for 3 d before the initiation of the experiment. After the start of the experiment, birds were fed the test diets for 7 d (adaptation period) prior to a 2-d collection period. Excreta were collected daily during the balance period and stored at -18 C. Afterwards, excreta were freeze-dried, equilibrated at ambient temperature for 24 h, weighed, ground, and stored in plastic bags for chemical analyses.

The APD values were calculated from the weighted average of apparent digestibilities of individual amino acids. Glycine was eliminated from this calculation because uric acid is degraded to glycine and ammonia during the acid hydrolysis of excreta (Soares *et al.*, 1971). The $AME_n$, APD, and starch digestibility values assigned to peas were calculated by assuming additivity of values assigned to basal and pea

fractions.

***Performance Trial***

The trial was designed to compare the performance of broiler chicks fed diets containing untreated, dehulled and micronized peas and to determine the response of broiler chicks fed micronized peas supplemented with lysine. Eleven isoenergetic and isonitrogenous diets were formulated. Diet 1 was a typical wheat-soybean meal diet and diets 2 to 11 contained peas replacing wheat and soybean meal. Except for those diets that contained dehulled peas (in which a correction was made for the amounts of hulls), the inclusion level of peas in all diets was 400 g/kg. Diet 11 was supplemented with 0.15% L-lysine to meet the digestible lysine level (calculated) of the diet containing untreated Impala peas (see footnote in Table 44). The compositions and calculated analyses of the diets are presented in Table 44.

Three hundred and thirty male day-old Arbor Acres broiler chicks were used. Housing and management of birds were as described for the digestibility trial with broiler chicks. From Day 1 to 3, the birds were fed commercial chick starter crumbles containing 210 g/kg CP. On Day 3, chicks were sorted into six weight groups from which they were allocated to experimental pens at six birds per pen. The pens were randomly assigned to 11 dietary treatments with five pens per treatment. The experiment lasted for a period of 14 d. Performance criteria examined included weight gain, feed consumption, and feed conversion ratio.

TABLE 44. Composition and calculated analysis (g/kg) of experimental diets, Experiment 10.

| Ingredients | Control | Impala[1] | | | Radley[1] | | | Sirius[1] | | | Impala |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Untreat. | Dehull. | Micro. | Untreat. | Dehull. | Micro. | Untreat. | Dehull. | Micro. | Micro. |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Peas | - | 400.0 | 364.0 | 400.0 | 400.0 | 360.0 | 400.0 | 400.0 | 356.0 | 400.0 | 400.0 |
| Soybean | 226.5 | 138.2 | 133.1 | 138.2 | 155.7 | 157.5 | 155.7 | 176.5 | 149.0 | 176.5 | 138.2 |
| Wheat | 690.5 | 345.9 | 360.0 | 345.9 | 333.9 | 344.4 | 333.9 | 281.2 | 333.6 | 281.2 | 344.4 |
| Limestone | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 | 19.5 |
| Biophos | 13.7 | 13.7 | 13.7 | 13.7 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 12.9 | 13.7 |
| DL-Met | 1.7 | 1.7 | 1.7 | 1.7 | 2.0 | 1.9 | 2.0 | 1.9 | 2.0 | 1.9 | 1.7 |
| L-Lysine | 1.6 | - | - | - | - | - | - | - | - | - | 1.5 |
| Vitamin mix[2] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral mix[3] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| Vegetable oil | 31.5 | 66.0 | 69.0 | 66.0 | 61.0 | 64.8 | 61.0 | 93.0 | 88.0 | 93.0 | 66.0 |
| Alpha cell | - | - | 24.0 | - | - | 24.0 | - | - | 24.0 | - | - |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| Calculated analysis, g/kg | | | | | | | | | | | |
| Energy, Mcal/kg | 3.04 | 3.01 | 3.04 | 301 | 3.04 | 3.04 | 3.04 | 3.01 | 3.01 | 3.01 | 3.04 |
| Protein | 222.7 | 221.2 | 222.5 | 221.2 | 222.1 | 222.7 | 222.1 | 222.6 | 222.1 | 222.6 | 222.4 |
| Calcium | 10.7 | 10.9 | 10.8 | 10.7 | 10.5 | 10.7 | 10.5 | 10.6 | 10.6 | 10.6 | 10.7 |
| Avail. P. | 4.5 | 4.7 | 4.6 | 4.7 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.5 | 4.7 |
| Lysine | 11.1 | 11.8 | 12.1 | 11.8 | 12.5 | 12.7 | 12.5 | 12.1 | 12.4 | 12.1 | 14.1 |
| Met | 5.1 | 5.1 | 5.1 | 5.1 | 5.0 | 5.1 | 5.0 | 5.1 | 5.1 | 5.1 | 5.1 |
| Met + Cys | 8.6 | 8.3 | 8.3 | 8.3 | 8.4 | 8.6 | 8.4 | 8.3 | 8.4 | 8.3 | 8.3 |

[1] Peas; [2,3] Same as Table 43.

The digestible lysine levels (calculated) for diets 2, 4 and 11 were 11.4, 10.6 and 11.5 g/kg, respectively.

*Statistical Analysis*

Data were analyzed using the General Linear Models (GLM) procedure of SAS® (SAS Institute, 1986). Treatment means were compared by Duncan's multiple range test (Duncan, 1955) and in some cases by orthogonal contrasts when $F$ values had probabilities less than 0.05.

## RESULTS AND DISCUSSION

*Composition of Peas*

Table 45 shows the chemical composition of untreated, dehulled, and micronized peas. Dehulling influenced ($P \leq 0.05$) the CP, starch, and NDF contents of peas. However, there was no effect ($P \geq 0.05$) of dehulling on moisture, ash, fat, calcium, and phosphorus contents. Micronization did not ($P \geq 0.05$) modify the chemical composition of peas. Moisture, CP, NDF, phosphorus contents were marginally decreased whereas the contents of ash and fat slightly increased. The effect on starch and calcium levels was not consistent. The amino acid profiles of untreated, dehulled, and micronized peas, expressed as grams per 16 g N, are presented in Table 46. The concentrations of several amino acids were higher in dehulled pea samples than in whole seeds. However, the effect of dehulling on the concentrations of most amino acids was not significant ($P \geq 0.05$). On the other hand, micronization reduced ($P \leq 0.05$) the concentrations of aspartic acid, glutamic acid, isoleucine, lysine, threonine, and tyrosine. Lysine concentration was decreased by 2.7%. In general, on a cultivar basis, micronization decreased total concentrations of amino acids by 3.3, 3.6, and 5.6% in Impala, Radley and Sirius peas,

TABLE 45. Chemical composition (g/kg) of untreated, dehulled and micronized peas.

| Parameters | Impala[1] | | | Radley[1] | | | Sirius[1] | | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Untreat[2] | Dehull | Micro | Untreat | Dehull | Micro | Untreat | Dehull | Micro | Untreat | Dehull | Micro | SEM |
| Moisture | 112.7[3] | 117.8 | 104.8 | 126.1 | 114.4 | 111.3 | 129.3 | 127.3 | 115.8 | 122.7a[4] | 119.8a | 110.6a | 4.12 |
| Protein | 245.2 | 272.7 | 238.3 | 232.2 | 252.6 | 227.6 | 231.9 | 269.3 | 226.2 | 236.4b | 264.9a | 230.7b | 4.91 |
| Ash | 30.6 | 28.4 | 35.4 | 25.8 | 29.0 | 30.6 | 29.5 | 28.4 | 31.0 | 28.6a | 28.6a | 32.3a | 1.25 |
| Fat | 14.3 | 15.5 | 20.4 | 20.1 | 24.1 | 28.5 | 12.4 | 15.6 | 19.0 | 15.6a | 18.4a | 22.6a | 2.72 |
| NDF[5] | 152.4 | 98.0 | 143.8 | 151.8 | 108.2 | 148.6 | 163.1 | 119.0 | 150.9 | 155.8a | 108.4b | 147.8a | 4.26 |
| Starch | 400.8 | 470.8 | 446.6 | 425.1 | 453.3 | 449.6 | 434.0 | 460.8 | 424.4 | 420.0b | 461.6a | 440.2ab | 7.90 |
| Calcium | 1.3 | 0.9 | 1.1 | 0.7 | 0.3 | 0.7 | 0.8 | 0.5 | 0.6 | 0.9a | 0.6a | 0.8a | 0.17 |
| Phospho. | 4.3 | 4.1 | 3.9 | 4.4 | 4.0 | 3.6 | 4.4 | 4.3 | 3.6 | 4.4a | 4.1a | 3.7a | 0.19 |

[1] Peas.
[2] Data for untreated peas were adapted from Igbasan and Guenter (1996a).
[3] Two observations per value,[4] Six observations per value.
[5] Neutral detergent fiber.
a b Means within the same row followed by the same letters are not significantly ($P \geq 0.05$) different.

TABLE 46. Amino acid profiles (g per 16 g N) of untreated, dehulled and micronized peas.

| AA | Impala[1] | | | Radley[1] | | | Sirius[1] | | | Statistical analysis | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Untreat[2] | Dehull | Micro | Untreat | Dehull | Micro | Untreat | Dehull | Micro | Untreat | Dehull | Micro | SEM |
| Ala | 4.2[3] | 4.0 | 3.9 | 4.4 | 4.3 | 4.5 | 4.4 | 4.3 | 3.7 | 4.3a[4] | 4.2a | 4.0a | 0.16 |
| Arg | 9.3 | 10.6 | 9.8 | 8.6 | 9.7 | 8.3 | 8.5 | 9.7 | 8.4 | 8.8a | 10.0a | 8.8a | 0.36 |
| Asp | 11.5 | 12.1 | 10.5 | 11.8 | 12.3 | 10.9 | 11.9 | 12.6 | 11.0 | 11.7b | 12.3a | 10.8c | 0.14 |
| Cys | 1.5 | 1.5 | 2.2 | 1.6 | 1.6 | 1.9 | 1.7 | 1.5 | 1.7 | 1.6b | 1.5b | 1.9a | 0.09 |
| Glu | 16.8 | 17.3 | 15.8 | 16.3 | 17.3 | 15.4 | 16.6 | 17.1 | 15.8 | 16.6b | 17.2a | 15.7c | 0.12 |
| Gly | 4.3 | 4.3 | 4.0 | 4.4 | 4.3 | 4.4 | 4.4 | 4.2 | 3.8 | 4.4a | 4.3a | 4.1a | 0.11 |
| His | 2.4 | 2.5 | 2.4 | 2.8 | 2.6 | 2.5 | 2.4 | 2.4 | 2.2 | 2.5a | 2.5a | 2.4a | 0.10 |
| Ile | 4.5 | 4.4 | 3.8 | 4.6 | 4.5 | 3.9 | 4.6 | 4.3 | 3.1 | 4.6a | 4.4a | 3.6b | 0.15 |
| Leu | 7.3 | 7.1 | 7.3 | 7.2 | 7.0 | 7.1 | 7.0 | 6.8 | 7.0 | 7.2a | 7.0a | 7.1a | 0.09 |
| Lys | 7.2 | 7.3 | 7.2 | 7.5 | 7.4 | 7.2 | 7.4 | 7.4 | 7.2 | 7.4a | 7.4a | 7.2b | 0.06 |
| Met | 1.2 | 1.2 | 1.2 | 0.9 | 0.8 | 1.2 | 0.9 | 0.9 | 1.3 | 1.0a | 1.0a | 1.2a | 0.09 |
| Phe | 4.9 | 4.8 | 4.9 | 4.8 | 4.9 | 4.9 | 4.8 | 4.8 | 5.1 | 4.8a | 4.8a | 5.0a | 0.05 |
| Pro | 4.3 | 4.2 | 4.4 | 4.4 | 4.4 | 3.8 | 4.3 | 4.3 | 4.5 | 4.3a | 4.3a | 4.2a | 0.13 |
| Ser | 4.8 | 4.8 | 5.4 | 4.8 | 4.8 | 5.5 | 4.9 | 4.9 | 5.0 | 4.8b | 4.8b | 5.3a | 0.09 |
| Thr | 3.7 | 3.7 | 3.3 | 3.9 | 3.9 | 3.5 | 3.9 | 3.8 | 3.4 | 3.8a | 3.8a | 3.4b | 0.06 |
| Tyr | 3.4 | 3.4 | 2.9 | 3.7 | 3.5 | 3.2 | 3.3 | 3.4 | 3.1 | 3.5a | 3.4a | 3.1b | 0.09 |
| Val | 4.8 | 4.9 | 3.9 | 4.7 | 4.6 | 4.7 | 4.8 | 4.8 | 4.1 | 4.8a | 4.8a | 4.2a | 0.15 |
| Total | 96.1 | 97.8 | 92.9 | 96.4 | 97.7 | 92.9 | 95.8 | 97.0 | 90.4 | 96.1a | 97.5a | 92.1b | 0.51 |

[1] Peas, [2] Same as Table 45, [3] Two observations per value, [4] Six observations per value.
abc Means within the same row followed by the same letters are not significantly ($P \geq 0.05$) different.
AA = Amino acids.

respectively. There were considerable variations in the chemical composition and amino acid concentrations among the three cultivars of peas evaluated.

Micronization and dehulling caused some changes in the chemical composition of peas. Changes in composition as a result of micronization have been reported for other grains. In barley and corn, Mercier (1971) and Lawrence (1973) showed that the micronization process decreased moisture and CP levels but increased fat content. Similar findings were reported by Savage and Clark (1988) and Douglas *et al.* (1991) for sorghum grains. Douglas *et al.* (1991) also observed that micronization caused minor alterations in the amino acid profiles of sorghum and corn. For both grains, lysine contents were decreased but cystine contents were increased; however, the effect on methionine level was not cosistent. Dehulling enriched the nutrient concentrations of peas. This result could be due to the removal of the dilution effect of fibre as evidenced from the reduction of the fibre (NDF) contents in dehulled seeds. The fibre content was decreased by 35.7, 28.7, and 27.0% in Impala, Radley, and Sirius peas, respectively. The increase in nutrient concentrations as a result of dehulling was similar to those observed previously with peas (Brenes *et al.*, 1993) and fababeans (Marquardt *et al.*, 1975).

***Digestibility Trials***

The $TME_n$ and TAAA data obtained from adult cockerels are presented in Table 47. Micronization induced a positive effect ($P \leq 0.01$) on $TME_n$ values irrespective of the pea cultivar. The improvement was 16.2, 9.5, and 19.1% for Impala, Radley, and Sirius peas, respectively. Regardless of cultivar, the effects of micronization on the availabilities

TABLE 47. True metabolizable energy (TMEn) and true amino acids availability (TAAA) of untreated and micronized peas.

| | Impala[1] | | Radley[1] | | Sirius[1] | | Untreat vs Micro | SEM |
|---|---|---|---|---|---|---|---|---|
| | Untreated | Micronized | Untreated | Micronized | Untreated | Micronized | | |
| TMEn, MJ/kg | 11.7d | 13.6ab | 12.6c | 13.8a | 11.0e | 13.1bc | ** | 0.16 |
| TAAA (%) | | | | | | | | |
| Alanine | 81.5b | 85.5a | 83.6a | 85.2a | 74.0c | 80.1b | ** | 0.59 |
| Arginine | 87.2c | 95.4a | 88.3c | 92.2b | 83.8d | 93.9ab | ** | 0.54 |
| Aspartic acid | 90.5b | 93.8a | 88.7bc | 90.5b | 82.5d | 87.6c | ** | 0.61 |
| Cystine | 69.2c | 73.0b | 72.4b | 75.6a | 57.3d | 70.8bc | ** | 0.62 |
| Glutamic acid | 89.5b | 95.7a | 89.9b | 91.2b | 84.3d | 87.5c | ** | 0.57 |
| Histidine | 89.4a | 89.8a | 82.4c | 84.4bc | 85.5b | 85.8b | ns[2] | 0.58 |
| Isoleucine | 84.6b | 91.6a | 84.1b | 90.1a | 75.7c | 84.9b | ** | 0.58 |
| Leucine | 86.7c | 93.5a | 87.5c | 90.6b | 78.8d | 86.8c | ** | 0.66 |
| Lysine | 86.1a | 76.4b | 77.2b | 73.8c | 73.4c | 71.9c | ** | 0.55 |
| Methionine | 80.4b | 82.1ab | 65.7d | 77.4c | 63.8e | 82.5a | ** | 0.53 |
| Phenylalanine | 82.2b | 87.8a | 82.4b | 83.7ab | 71.9c | 76.0c | * | 1.25 |
| Proline | 77.8bc | 87.6a | 78.2bc | 79.3b | 69.1d | 76.9c | ** | 0.57 |
| Serine | 84.8a | 85.9a | 86.8a | 89.1a | 74.5b | 86.0a | ** | 1.38 |
| Threonine | 80.3b | 81.7ab | 81.6ab | 85.0a | 70.3c | 78.9b | ** | 0.99 |
| Tyrosine | 83.5a | 86.5a | 84.3a | 87.1a | 72.2b | 73.8b | ns | 1.35 |
| Valine | 81.9bc | 90.2a | 82.7bc | 84.9b | 70.7d | 79.0c | ** | 1.39 |
| Mean | 83.5ab | 87.3a | 82.2b | 85.0ab | 74.2c | 81.4b | ** | 1.00 |

[1] Peas.

a-e Means within the same row followed by the same letters are not significantly ($P \geq 0.05$) different

* $P \leq 0.05$; $P \leq 0.01$; [2] Not significant.

of most amino acids were highly significant ($P \leq 0.01$). There was no effect ($P \geq 0.05$) of micronization on the availabilities of histidine and tyrosine. When compared with untreated peas, the overall mean availabilities of amino acids across the three pea cultivars were greatly affected ($P \leq 0.01$) by micronization. Except for lysine histidine, and tyrosine, the availabilities of all other amino acids in peas were improved by micronization. The availability of lysine was decreased by 11.3, 4.4 and 2.0% for Impala, Radley, and Sirius, respectively. The decrease was significant ($P \leq 0.05$) for Impala and Radley but not significant ($P \geq 0.05$) for Sirius peas. The availability of methionine was only slightly increased ($P \geq 0.05$) for Impala (2.1%), but substantially increased ($P \leq 0.05$) for Radley and Sirius peas (17.8% and 29.3%, respectively). In general, on cultivar basis, the mean availabilities of amino acids were not higher ($P \geq 0.05$) for micronized Impala and Radley (4.6% and 3.4%, respectively) and moderately higher ($P \leq 0.05$) for micronized Sirius (9.7%).

Compared with untreated peas, micronization had a profound effect ($P \leq 0.01$) on the $AME_n$ APD, and starch digestibility values of peas in young broiler chicks (Table 48). Dehulling also affected the $AME_n$ ($P \leq 0.01$), APD and starch digestibility ($P \leq 0.05$) values of peas; however, dehulling and micronization affected ($P \leq 0.01$) these parameters with different magnitude. Irrespective of cultivar, micronization substantially improved $AME_n$, APD and starch digestibility values. The increments ranged from 19.4 to 31.3, 8.5 to 33.6, and 12.2 to 22.3% for $AME_n$, APD, and starch digestibility, respectively. In contrast, dehulling produced marginal improvements in $AME_n$ values of Impala (yellow-seeded) (3.0%) and Radley (green-seeded) (4.9%) whereas the $AME_n$ value of Sirius

TABLE 48. Apparent metabolizable energy ($AME_n$), apparent protein digestibility (APD) and starch digestibility of untreated, dehulled and micronized peas, Experiment 9.

| Treatments | $AME_n$, Mcal/kg | APD, % | Starch digestibility, % |
|---|---|---|---|
| Untreated MP | 10.1c | 76.7bc | 84.1c |
| Dehulled MP | 10.4bc | 75.3cd | 85.8c |
| Micronized MP | 12.1a | 83.2a | 95.2a |
| Untreated RD | 10.3bc | 71.5ed | 86.1c |
| Dehulled RD | 10.8b | 68.5e | 87.2bc |
| Micronized RD | 12.3a | 80.0ab | 96.6a |
| Untreated SR | 8.3d | 60.1f | 72.6e |
| Dehulled SR | 10.3bc | 76.2bc | 81.4d |
| Micronized SR | 10.9b | 80.3ab | 88.8b |
| SEM | 0.21 | 1.55 | 0.87 |
| Contrast | | P Values | |
| Untreated vs Dehulled | ** | * | ns[1] |
| Untreated vs Micronized | ** | ** | ** |
| Dehulled vs Micronized | ** | ** | ** |

MP = Impala; RD = Radley; SR = Sirius.
a-f Means within the same column followed by the same letters are not significantly ($P \geq 0.05$) different.
* $P \leq 0.05$; ** $P \leq 0.01$; [1] Not significant.

(brown-seeded) was substantially improved (24.1%). The results of APD also showed that dehulling resulted in only a slight decrease in APD values of Impala (- 1.8%) and Radley (- 4.2%) but dramatically increased the APD value of Sirius peas (26.8%). Significant improvement in starch digestibility of Sirius peas similar to those in AMEn and APD values was also observed. Regardless of processing, the $AME_n$, APD, and starch digestibility values were higher ($P \leq 0.05$) in Impala and Radley peas than in the Sirius peas. This finding was also true for $TME_n$ and TAAA values. The $AME_n$ and starch digestibility values were similar ($P \geq 0.05$) for both Impala and Radley. All cultivars of peas evaluated exhibited differences ($P \leq 0.05$) in their protein digestibility.

The improvement in $TME_n$ and $AME_n$ values observed in this study is in agreement with earlier reports that demonstrated that heat treatment improved ME value of peas in chickens. Longstaff and McNab (1987) and Carre *et al.*, (1991) reported significant improvement in ME of peas as a result of autoclaving and steam pelleting. An improvement in the digestible energy value of barley and corn for growing pigs and soybeans for growing rats as a result of micronization was also reported by Lawrence (1973) and Hutton and Foxcroft (1975). Application of micronization resulted in an increase in AA availabilities and apparent protein digestibility. The increase revealed that some form of heat treatment is required to inactivate protease inhibitors present in peas (Griffiths, 1984) and to alter the three-dimensional structure of plant proteins to allow the protein to be more susceptible to enzyme hydrolysis (Nordheim and Coon, 1984). In studying the effect of micronization on faba beans, McNab and Wilson (1974) found that micronized faba beans had their trypsin inhibitor activity reduced by 91.6%. The

reduction in lysine availability observed in this study may result from the formation of Maillard reaction products, which are condensation and polymerization products of AA like lysine with sugar aldehyde groups (Bjarnason and Carpenter, 1970). Maillard products are regarded as fiber and are resistant to enzyme degradation. Pancreatic proteases (trypsin and chymotrypsin) are rich in methionine and cystine and an excessive endogenous loss of these AA due to stimulation of pancreatic enzyme production (resulting from the actions of protease inhibitors) may lead to a low availability of methionine (Kempen, 1993). The inactivation of these inhibitors may explain, in part, the increase in the availability of methionine as well as cystine.

It is evident that infrared treatment (micronization) was able to increase the digestibility of pea starch. The beneficial effect of micronization could be as a result of its influence on gelatinization or disruption of intermolecular bonds in pea starch. According to Moran (1982) difficulties in starch digestion with fowl can be alleviated by heat treatments that initiate granule gelatinization. Similarly, McNeill *et al.* (1975) found that gelatinized or disrupted starch is more rapidly degraded by enzymes than raw starch. This improvement in starch digestibility agrees very well with earlier reports by Lawrence (1973) and McNab and Wilson (1974), which showed that micronization increased *in vitro* starch availability in cereal grains and faba beans.

Although dehulling removes a substantial amount of fibre, feeding dehulled peas to broiler chicks did not improve protein digestibility but slightly increased the $AME_n$ and starch digestibility values of Impala (yellow-seeded) and Radley (green-seeded) peas. These effects may be attributed to the increased protease inhibitor content that is known

to be present in the cotyledons at much higher concentration than in the hulls (Poel *et al.*, 1989). The slight increase in starch digestibility when the hulls were excluded may be due to a better enzyme to substrate contact. Longstaff and McNab (1987) have demonstrated that digestion of starch is being influenced by its accessibility to the digestive enzymes. The authors also observed improvement in *in vitro* starch digestion when pea hulls were removed from the incubation medium. Dehulling of Sirius peas (brown-seeded) increased the $AME_n$, protein, and starch digestibility values. This positive response can be substantiated from the fact that the hull fraction of peas contains phenolic compounds (tannins) that interfere with nutrient digestion and utilization. This cultivar has been shown to contain appreciable quantity of tannins (41.0 g/kg - catechin equivalents) that are located in the hulls whereas the other two cultivars (Impala and Radley) are tannin-free (< 1.0 g/kg) (Igbasan *et al.*, 1994; Igbasan and Guenter, 1996a). Similar studies with tannin-containing Maple pea cultivar (Brenes *et al.*, 1993) and faba beans (Marquardt and Ward, 1979) showed that dehulling was effective in improving their nutritive value.

The improvements in $AME_n$ values of peas correspond to the improvements in protein and starch digestibilities as earlier reported by Carre *et al.* (1987) and Conan and Carre (1989). The differences in ME, protein, and starch digestibility values among the pea cultivars used in this study have been reported earlier (Igbasan and Guenter, 1996a).

***Performance Trial***

The results of the 2-wk performance trial of broiler chicks fed untreated, dehulled

and micronized peas are presented in Table 49. Broiler chicks fed diets containing 400 g/kg of untreated Impala, Radley, and Sirius peas had lower weight gains than those birds fed the wheat-soybean control diet; however, this was only significant ($P \leq 0.05$) with Sirius peas. The corresponding FCR was also affected ($P \leq 0.05$). Compared with untreated peas, dehulling as well as micronization produced significant effects on weight gains ($P \leq 0.01$) and FCR ($P \leq 0.01$) but not on feed consumption. Both dehulling and micronization produced different effects on feed consumption ($P \leq 0.05$) and FCR ($P \leq 0.01$). Birds fed dehulled Sirius and Radley peas consumed more feed than their counterparts fed micronized peas. On the other hand, birds fed micronized peas had better feed conversion than those fed dehulled peas.

The poor performance of chicks fed untreated peas is in agreement with our earlier reports (Igbasan and Guenter, 1996a). In addition, we showed differences in performance of broiler chicks fed different cultivars of peas varying in nutrient concentrations and coat colour that were similar to those observed among cultivars used in this study. Compared with those birds fed untreated peas, there were improvements in weight gains and FCR of birds fed dehulled Impala (5.3%, 5.5%), Radley (6.3%, 3.0%) and Sirius (9.8%, 5.7%) peas. The magnitude of improvements is higher in Sirius (brown-seeded) than in Impala (yellow-seeded), and Radley (green-seeded) peas. This result could be related to the tannin content in this cultivar. The magnitude of improvements in performance follows closely the improvements in nutrient availabilities observed in the digestibility trial with chicks.

Regardless of the pea cultivar, micronization improved weight gains and feed

TABLE 49. Weight gain, feed consumption and feed conversion ratio (FCR) of broiler chicks (3-17 d) fed untreated, dehulled and micronized peas, Experiment 10.

| Treatments | Weight gain, g | Feed consumption, g | FCR |
|---|---|---|---|
| 1 Wheat-soy | 339.6bcd | 531.7b | 1.57cd |
| 2 Untreated MP | 329.2cde | 543.2ab | 1.65e |
| 3 Dehulled MP | 346.7bc | 542.3ab | 1.56cd |
| 4 Micronized MP | 358.0ab | 529.7b | 1.48ab |
| 5 Untreated RD | 322.1de | 536.7b | 1.67e |
| 6 Dehulled RD | 342.5cde | 553.2ab | 1.62de |
| 7 Micronized RD | 354.6b | 542.2ab | 1.53bc |
| 8 Untreated SR | 314.5e | 546.8ab | 1.74f |
| 9 Dehulled SR | 345.2bc | 565.5a | 1.64e |
| 10 Micronized SR | 351.4bc | 538.0b | 1.53bc |
| 11 Micronized MP + Lysine | 377.0a | 536.6b | 1.42a |
| SEM | 7.19 | 8.22 | 0.023 |
| Contrast | | P Values | |
| Untreated vs Dehulled | ** | ns[1] | ** |
| Untreated vs Micronized | ** | ns | ** |
| Dehulled vs Micronized | ns | * | ** |

MP = Impala; RD = Radley; SR = Sirius.
a-f Means within the same column followed by the same letters are not significantly ($P \geq 0.05$) different.
* $P \leq 0.05$; ** $P \leq 0.01$; [1] Not significant.

conversion efficiency of broiler chicks. The respective improvements in weight gains and feed conversion, above untreated peas, were: 8.7% and 10.3%, 10.1% and 8.4% and 11.7% and 12.1% for Impala, Radley, and Sirius peas, respectively. Addition of lysine to micronized Impala peas improved ($P \geq 0.05$) weight gains and FCR by 5.3 and 4.1% above un-supplemented micronized Impala peas. Compared with untreated Impala peas, micronization coupled with lysine supplementation improved ($P \leq 0.05$) weight gain and feed conversion of broiler chicks by 14.5 and 13.9%, respectively. Both diets had similar digestible lysine level. Feed consumption was not affected by the processing methods, however, birds fed the dehulled Sirius peas did have a higher ($P \leq 0.05$) feed consumption.

The better energy, protein, and starch utilization of micronized peas shown in digestibility trials contributed to a faster growth rate and better feed conversion in broiler chicks. McNab and Wilson (1974) and Douglas *et al.* (1991) have shown in their studies that the improvements in weight gains and feed conversion of chicks fed micronized faba beans and sorghum were related to the increase in starch availability and AMEn values of these grains. The biological availability of lysine in micronized peas may be a concern as evidenced from the improvement in performance of chicks fed micronized peas supplemented with 0.15% L-lysine. Some amount of lysine might have been involved in cross-linking reactions with either carbohydrate or other amino acids in the process of heating. "Bound" lysine is not digestible and in turn, is unavailable to the animals. The TAAA data (Table 5) indicate that up to 11% of lysine may be involved in these cross-link formations.

In summary, dehulling increased protein, starch and total amino acid concentration but decreased fibre content. On the other hand micronization decreased moisture, protein and fibre contents as well as the total concentrations of amino acid. The $TME_n$, $AME_n$, TAAA, APD, and starch digestibility values were also improved by micronization. Chicks fed micronized peas performed better than chicks fed the untreated peas or the wheat-soybean control diet. Addition of lysine to the diet containing micronized peas further improved the performance of chicks. The improvement may be related to the adverse effect of micronization on the availability of lysine. Dehulling was more beneficial when applied to tannin-containing peas.

## MANUSCRIPT 7

# THE INFLUENCE OF MICRONIZATION, DEHULLING, AND ENZYME SUPPLEMENTATION ON THE NUTRITIONAL VALUE OF PEAS FOR LAYING HENS

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**ABSTRACT** Two experiments (experiments 11 & 12) were conducted to determine the effect of micronization (infrared heat treatment), dehulling or enzyme supplementation on the nutritive value of three cultivars (Impala, Radley and Sirius) of peas in laying hens. In experiment 11, a total of 280 White Leghorn hens were fed diets containing micronized and dehulled peas for a period of 12 wk. The dietary inclusion of peas was 600 g/kg (correction was made for the amounts of hulls in those diets that contained dehulled peas). Egg production ($P \leq 0.05$), feed conversion ($P \geq 0.05$) and egg mass output ($P \leq 0.05$) were lower for birds fed untreated peas. Daily feed intake, egg weight, and albumen quality were not affected. Egg production, feed conversion and egg mass output were similar for layers fed diets containing micronized peas and those fed the wheat-soybean control diet. Except for daily feed intake which was reduced ($P \leq 0.05$), dehulling did not affect ($P \geq 0.05$) the feeding value of peas. The efficacy of dietary inclusion of crude enzyme was evaluated in experiment 12 (8 wk). The enzyme investigated was pectinase and was included in the experimental diets at 0, 50 and 100 U/kg, and fed to 252 layers. The inclusion level of peas in these diets was 650 g/kg. There was no effect ($P \geq 0.05$) of enzyme supplementation on all production traits. The egg production, feed conversion and egg mass output were 81.9, 83.6 and 83.0%; 1.61, 1.59 and 1.59 and 50.0, 51.2, and 50.3 at 0, 50 and 100 U/kg levels, respectively. It can be concluded that of the treatments tested only micronization had a positive effect on the feeding value of peas for laying hens.

**Key words:** peas, micronization, dehulling, enzyme supplementation, layer

# INTRODUCTION

The use of peas (*Pisum sativum* L.) in commercial laying hen diets is very limited. Substitution of high amounts of wheat/barley and soybean meal with peas has been associated with decreased egg production and feed utilization (Igbasan and Guenter 1996c; Davidson *et al.* 1981). The predominant causal factors are considered to be protease inhibitors (Griffiths 1984), tannins (Griffiths 1981; Lindgren 1975), lectins (Bender 1983) and possibly non-starch polysaccharides (Saini 1989; Longstaff and McNab 1987).

In comparison to most cereal grains, pea seeds are only slightly lower in starch. As a nutrient, starch is the greatest single dietary source of energy. However, pea starch is less susceptible to enzyme hydrolysis than starch in any of the cereal grains (Longstaff and McNab 1987) because of strong intermolecular bonds (Colona and Mercier 1979). Processing procedures that eliminate or inactivate antinutritve substances from peas and alter starch structure to improve accessibility of starch granules to enzyme hydrolysis offer promise of improving the nutritive value of peas for laying hens.

Micronization, a name given to a dry-heat process using infrared electromagnetic short waves produced by burning industrial propane over ceramic tile or nichrome wire elements to heat grains was described first by Mercier (1971) and later by Lawrence (1973) and McNab and Wilson (1974). Lawrence (1973) and Douglas *et al.* (1991) reported that micronization improved the nutritive value of cereal grains for growing pigs and chickens. The same improvement has been reported for full fat soybeans (Hutton and Foxcroft 1975) and faba beans (*Vicia faba*) (McNab and Wilson 1974).

Peas contain substantial amounts of pectic polysaccharides (Brillouet and Carre

1983) which may have antinutritive effects for laying hens. According to Cleophas *et al.* (1995), pectins are able to make viscous solutions thereby increasing digesta viscosity, the mechanism by which soluble non-starch polysaccharides exert their antinutritive effects. Pectins are susceptible to endo-galactouranase (pectinase) degradation (Annison and Choct 1993). However, these polysaccharides are highly branched and this makes the backbone not readily accessible for naturally occurring endo-hydrolyzing enzyme. Exogenous pectinase may be required to hydrolyze the backbone.

Few reports are available on the use of enzymes in legume based diets. These reports are limited to broiler chickens and most of them did not show significant beneficial effects of adding enzymes to legume based diets. There was no major improvement in growth rate and feed utilization of broiler chickens when fed diets containing soybeans (Anderson and Warnick 1964), field beans (*Vicia faba*) (Castanon and Marquardt 1989) and peas (Brenes *et al.* 1993) supplemented with enzymes. Our recent study (Igbasan and Guenter, 1996a) showed that supplementation of diets containing yellow, green, and brown peas with pectinase significantly improved weight gains and feed consumption of broiler chickens but feed conversion was not affected, whether addition of enzyme to pea-based diets would produce the same or different response in laying hens is not known.

As part of a wider study on the evaluation and enhancement of the nutritive value of peas in poultry diets, the study described here was performed to determine the extent to which micronization, dehulling or enzyme supplementation would improve the utilization of peas by laying hens.

# MATERIALS AND METHODS

## *Plant Materials and Processing Conditions*

Three cultivars of peas, Impala (yellow-seeded), Radley (green-seeded) and Sirius (brown-seeded) used for this study were locally grown. Peas were micronized (Infrared heated) at a temperature between 110 and 115 C for 55 s. in a micronizer[1] located at InfraReady Products Limited, Saskatoon, Saskatchewan, Canada. Micronized peas were also subjected to a roll flaking process before ground for use. Dehulling was accomplished with the aid of a roller mill and the hulls were separated from the cotyledons by air classification. The chemical compositions of untreated, micronized and dehulled pea samples have been reported (Igbasan and Guenter 1996c).

## *Experiment 11*

Two hundred and eighty Single Comb White Leghorn (SCWL) pullets of Shaver White strain were used to evaluate the feeding value of micronized and dehulled peas in a 12 wk experiment. The birds were housed in colony cages (40 x 25 cm) at 2 birds per cage. The cages were equipped with Hart cup waterers and trough feeders. The hens were housed in an environmentally controlled house at a temperature of about 25 C and 16 h of light/d.

Ten isoenergetic and isonitrogenous diets were formulated (Table 50). Except for

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[1]Micronizing Company, UK., Framlingham, Suffolk, 1P13 9PT, England.

those diets that contained dehulled peas, where correction was made for the amounts of hulls (546 g/kg pea cotyledons), the inclusion level of peas in other diets was 600 g/kg. The diets were fed to pullets when daily egg production per pullet reached 70%. Each diet was randomly replicated seven times with four birds per replicate. The duration of the experiment was 84 d and was divided into three periods of 28 d each.

Eggs were collected and recorded daily for each replicate. Feed consumption was determined on a replicate basis by weighing feed at the beginning and at the end of each period. Hens were weighed individually at the start and at the end of the experiment. Mortality was recorded as it occurred. On the last 3 consecutive d of each period, all eggs collected were identified by cage number and kept for egg quality determinations: weight, albumen height, yolk color and shell thickness. Albumen height was measured using an electronic albumen height gauge[2], yolk colour was determined using the Roche yolk colour fan[3] (15, dark orange; 1, light pale) and shell thickness was measured using an Ames micrometer[4]. Egg mass output (rate of lay x egg weights) and feed conversion (kg feed ÷ dozen eggs) were calculated from the data.

***Experiment 12***

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[2]Queensboro Instuments, 645 Briewood, Ottawa, Canada.

[3]Hoffman-La Roche Ltd., Basel, Switzerland

[4]B.C. Ames, Co., Waltham, Mass.

TABLE 50. Composition and calculated analysis of experimental diets, Experiment 11.

| Ingredients | Control | Impala | | | Radley | | | Sirius | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Untreat. | Dehull. | Micro. | Untreat. | Dehull. | Micro. | Untreat. | Dehull. | Micro. |
| | g/kg | | | | | | | | | |
| Peas | - | 600.0 | 546.0 | 600.0 | 600.0 | 540.0 | 600.0 | 600.0 | 534.0 | 600.0 |
| Wheat | 605.9 | 138.9 | - | 138.9 | 200.2 | 213.6 | 200.2 | 242.0 | 30.0 | 242.0 |
| Barley | 100.0 | 95.0 | 268.8 | 95.0 | 57.0 | 65.0 | 57.0 | - | 244.0 | - |
| Soybean | 145.0 | - | - | - | - | - | - | - | - | - |
| Limestone | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 | 87.0 |
| Biophos | 10.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 | 9.0 |
| Vitamin[1] | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 | 10.0 |
| Mineral[2] | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| L-Lysine | 1.1 | - | - | - | - | - | - | - | - | - |
| DL-Meth. | 1.0 | 1.1 | 1.2 | 1.1 | 1.8 | 1.4 | 1.8 | 1.5 | 1.5 | 1.5 |
| Vegetable oil | 35.0 | 54.0 | 41.0 | 54.0 | 30.0 | 37.0 | 30.0 | 45.5 | 53.0 | 45.5 |
| Alpha cell | - | - | 32.0 | - | - | 32.0 | - | - | 26.5 | - |
| Total | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 | 1000.0 |
| Calculated analysis | | | | | | | | | | |
| TMEn, Mcal/kg | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.87 | 2.84 | 2.84 | 2.84 |
| Protein | 183.6 | 184.7 | 186.0 | 183.6 | 182.6 | 182.8 | 182.6 | 181.8 | 182.9 | 181.8 |
| Calcium | 35.5 | 35.6 | 35.5 | 35.5 | 35.5 | 35.5 | 35.5 | 35.3 | 35.5 | 35.3 |
| Avail. P. | 3.5 | 3.6 | 35.0 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 | 3.5 |
| Lysine | 8.2 | 10.1 | 10.9 | 10.1 | 10.6 | 10.8 | 10.6 | 9.3 | 10.7 | 9.3 |
| Met. | 3.8 | 3.9 | 3.9 | 3.8 | 3.9 | 3.9 | 3.9 | 3.8 | 3.9 | 3.8 |
| Met. + Cys. | 6.7 | 6.5 | 6.7 | 6.5 | 6.6 | 6.7 | 6.6 | 6.4 | 6.6 | 6.4 |

[1] Amount supplied per kilogram of diet were: Vitamin A, 8250 IU; Cholecalciferol, 1000 IU; Vitamin E, 5.46 IU; Vitamin $B_{12}$, 0.12 mg; Riboflavin, 2.2 mg; Niacin, 6.6 mg; Ca-pantothenate, 4.4 mg; Choline chloride, 110 mg; DL-methionine, 500 mg.
[2] Amount supplied per kilogram of diet were: Manganese, 110 mg; Zinc, 55 mg; Iodized salt, 4780 mg.

In this experiment, the efficacy of dietary inclusion of a crude enzyme preparation in diets in which peas constituted a major dietary constituent was studied. The enzyme investigated was pectinase. It was supplied by Finnfeeds International[5] and contained 3,500 U/g of pectinase activity (as determined by the manufacturer) and the recommended dietary inclusion level was 50 U/kg.

A total of two hundred and fifty two SCWL pullets (Shaver White strain) were randomly distributed among 9 dietary treatments arranged in a 3 x 3 factorial. Each treatment group consisted of 7 replicates of 4 birds each. Housing and management of birds were as described in experiment 11. The dietary treatments were as follows: (1) Impala (650 g/kg) pea diet (Basal 1); (2) Basal 1 plus 50 U/kg pectinase; (3) Basal 1 plus 100 U/kg pectinase; (4) Radley (650 g/kg) pea diet (Basal 2); (5) Basal 2 plus 50 U/kg pectinase; (6) Basal 2 plus 100 U/kg pectinase; (7) Sirius (650 g/kg) pea diet (Basal 3); (8) Basal 3 plus 50 U/kg pectinase and Basal 3 plus 100 U/kg pectinase. The 3 basal diets were formulated to meet NRC requirements (NRC, 1994) for laying hens. The compositions and calculated analyses of the basal diets are presented in Table 51. Because our basal diets contained moderate levels of barley (about 200 g/kg) and this might make it difficult to assess the efficacy of pectinase targeting the pectic polysaccharides in peas, Avizyme 1100 (supplied by Finnfeeds International Ltd.) containing 100 U/g β-glucanase (EC 3.2.1.6) (which would be active against β-glucans of barley), 300 U/g xylanase (EC 3.2.1.8) and 800 U/g protease, was added to all diets following manufacturer's

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[5]Finnfeeds International Ltd., Marborough, Wiltshire, UK.

TABLE 51. Composition and calculated analysis of basal, Experiment 12.

| Ingredients | Impala | Radley | Sirius |
|---|---|---|---|
| | | g/kg | |
| Peas | 650.0 | 650.0 | 650.0 |
| Barley | 203.0 | 207.5 | 202.0 |
| Limestone | 87.0 | 87.0 | 87.0 |
| Biophos | 9.0 | 9.0 | 9.0 |
| DL-Methionine | 1.0 | 1.5 | 1.5 |
| Vitamin[1] | 10.0 | 10.0 | 10.0 |
| Mineral[2] | 5.0 | 5.0 | 5.0 |
| Vegetable oil | 35.0 | 30.0 | 35.5 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| | | | |
| Calculated analysis | | | |
| TMEn, Mcal/kg | 2.70 | 2.80 | 2.70 |
| Protein | 187.7 | 180.2 | 179.5 |
| Calcium | 35.6 | 35.4 | 35.3 |
| Avail. P. | 3.6 | 3.6 | 3.6 |
| Lysine | 10.8 | 10.9 | 9.8 |
| Met. | 3.8 | 3.7 | 3.8 |
| Met. + Cys. | 6.5 | 6.3 | 6.4 |

[1, 2] Same as Table 50.

recommendation.

The experiment was initiated when hen-day egg production was about 80%. The duration of the experiment was 56 d or 2 periods of 4 wk each. Initial and final body weights of hens were recorded at the beginning and at the end of the experiment. Feed consumption, egg production and incidence of mortality were recorded on a replicate basis. All eggs laid on the last 3 d of each period were identified by cage number and were used for exterior and interior egg quality determinations. Average egg weight was obtained for each replicate. Albumen height, yolk colour and shell thickness were determined as described in Experiment 11.

***Statistical Analysis***

All data were subjected to analysis of variance using the General Linear Models (GLM) procedure of SAS® (SAS Institute 1986), as a split-plot design but with a 3 x 3 factorial arrangement of treatments in experiment 12 ( 3 pea cultivars and 3 levels of enzyme addition, 0, 50, and 100 $\mu$/kg diet). Treatment differences obtained upon statistical analyses were compared using Duncan multiple range test (Duncan 1955).

## RESULTS AND DISCUSSION

***Experiment 11***

The main effects of dietary treatments with experimental period and their interactions on performance traits are shown in Table 52. There was a significant effect ($P \leq 0.05$) of treatments on egg production, feed intake, egg weight, egg mass output,

TABLE 52. Degrees of significance, *F* test, of treatments and periods and their interactions on performance traits of laying hens fed untreated, dehulled and micronized peas, Experiment 11.

| Source of variation | Probability values | | |
|---|---|---|---|
| | Treatment | Period | Treatment by Period |
| Rate of lay, % | ** | ** | NS |
| Daily feed intake, g/bird | ** | NS | NS |
| Feed conversion, kg/doz. | NS | NS | NS |
| Egg weight, g | *** | *** | NS |
| Egg mass output[1], g/h/d | *** | ** | NS |
| Yolk colour score | *** | NS | NS |
| Albumen height, mm | * | NS | NS |
| Shell thickness, mm x $10^{-2}$ | ** | *** | NS |
| Body weight change, kg | NS | NA | NA |

* Effect significant at $P \leq 0.05$.
** Effect significant at $P \leq 0.01$.
*** Effect significant at $P \leq 0.001$.
NS = Not significant; NA = Not applicable.
[1] Rate of lay times average egg weight.

yolk colour, albumen height, shell thickness but not on feed conversion and body weight. The effect of experimental period was only significant ($P \leq 0.05$) for egg production, egg weight and shell thickness. Egg production and egg weight increased over the course of the experimental period but shell thickness decreased. No effect of treatment by period interactions was observed on all performance traits, which suggests that the effect of treatment with respect to time (period) was the same.

Table 53 gives a summary of the performance data of laying hens fed untreated, micronized and dehulled peas. Birds fed diets containing 600 g/kg of untreated Impala, Radley and Sirius peas had lower egg production and egg mass output than those fed the wheat-soybean control diet. However, the effect on egg production was not significant for Impala peas. The corresponding feed conversion was also poor although this was not significant. Daily feed intake, egg weight, albumen height, body weight were not affected. Shell thickness was decreased for Impala but it was not affected by Radley or Sirius peas. Egg yolk colour was improved ($P \leq 0.05$) when peas were incorporated into the diet of layers. Dehulled and untreated peas gave similar results except that the birds fed dehulled peas had a reduced daily feed intake. Birds fed diets containing micronized peas had similar egg production, feed conversion and egg mass output compared to birds fed the wheat-soybean control diet. Micronization did not alter other performance traits. Mortality rates did not appear to be related to treatments applied.

The results of this experiment confirm the adverse effects of high amounts of raw peas in laying hen diets reported previously (Igbasan and Guenter, 1996c; Moran *et al.*, 1968). The positive influence of peas on yolk colour observed in this study may be

TABLE 53. Performance of laying hens fed untreated, dehulled and micronized peas, Experiment 11.

| Treatment | Rate of lay, % | Daily feed intake, g/bird | Feed conv., kg/doz. | Egg weight, g | Egg mass output[1], g/h/d | Yolk colour score[2] | Albumen height, mm | Shell thickness, mm x $10^{-2}$ | BWC, kg | Mortality rate, % |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 89.6a | 104.9ab | 1.42a | 60.9ab | 54.6a | 2.6d | 9.1a | 36.2ab | 0.07a | 3.6 |
| Untreated Impala[3] | 85.1abcd | 106.1a | 1.51a | 60.4ab | 51.4bc | 5.3c | 9.0ab | 34.4c | 0.05a | - |
| Dehulled Impala | 84.7abcd | 101.4c | 1.50a | 58.5b | 49.7bc | 5.5bc | 8.9ab | 35.3abc | 0.05a | 3.6 |
| Micronized Impala | 88.2ab | 104.0abc | 1.42a | 59.8ab | 52.7ab | 5.3c | 8.7ab | 34.7bc | 0.07a | - |
| Untreated Radley[3] | 82.4cd | 103.3abc | 1.51a | 60.3ab | 49.7bc | 6.0a | 8.8ab | 36.5a | 0.04a | - |
| Dehulled Radley | 82.1d | 101.8bc | 1.49a | 59.7ab | 49.0c | 6.3a | 8.7ab | 36.3ab | 0.05a | - |
| Micronized Radley | 87.0abcd | 105.6a | 1.46a | 60.7ab | 52.8ab | 6.1a | 8.9ab | 35.5abc | 0.05a | 3.6 |
| Untreated Sirius[3] | 83.3cd | 105.0ab | 1.52a | 59.7ab | 49.7bc | 5.8b | 8.9ab | 35.0abc | 0.05a | 3.6 |
| Dehulled Sirius | 83.8bcd | 102.7abc | 1.47a | 60.9ab | 51.0bc | 5.9b | 8.5b | 35.9abc | 0.04a | - |
| Micronized Sirius | 87.6abc | 106.0a | 1.45a | 62.0a | 54.3a | 5.6bc | 9.1a | 35.3abc | 0.05a | - |
| SEM | 1.67 | 1.53 | 0.033 | 0.74 | 1.05 | 0.09 | 0.15 | 0.54 | 0.013 | ND |

BWC = Body weight change; ND = Not determined..
[1] Rate of lay by average egg weight.
[2] Egg yolk colour based on Roche colour fan: 15, dark orange; 1, light pale yellow.
[3] Peas.
abcd Means within column followed by different letters differ significantly ($P \leq 0.05$).

related to the quantity of xanthophylls in peas, but at present there is no information on the xanthophyll contents of peas. Our previous work (Igbasan and Guenter, 1996c) showed a progressive improvement in yolk colour as the level of peas in laying hen diet was increased. Lack of response to dehulling observed here may be related to the increased content of thermolabile protease inhibitor which are known to be present in the cotyledons of peas at a much higher concentration than in the hulls (Valdebouze *et al.*, 1980). A similar study by Davidson (1980b) with field bean (*Vicia faba* cult. Pavane) rich in proanthocyanidines showed that there was no apparent beneficial effect resulting from feeding dehulled beans to laying hens.

With the application of infrared heat treatment (micronization) to peas, productivity of laying hens was equal to that of birds fed the wheat-soy control diet. The beneficial effects derived from this heat-processing arise, at least in part, from the destruction of antinutritive substances such as trypsin inhibitor and haemagglutinin activities in peas. McNab and Wilson (1974) and Davidson (1980b) reported a reduction in trypsin inhibitor and haemagglutinin activities in field beans (*Vicia faba*) as a result of micronization. It is also possible that heating altered or disrupted the structure of pea protein and starch thereby making these nutrients more susceptible to enzyme attack. Our recent study (Igbasan and Guenter 1996d) and that of McNab and Wilson (1974) have shown that infrared heat treatment improved apparent metabolizable energy, protein and starch digestibility values of peas and field beans in broiler chicks. This may also apply to laying hens. The improvement in nutrient utilization would explain the improvement in laying performance we observed here. In agreement with the current findings, Davidson

(1980a) reported improvements in egg production of hens fed micronized peas. However, the magnitude of improvement reported by this author was much higher than the one we observed in this study, 4.8 (average for the three types of peas) vs 20.0% above untreated peas. It should be noted that in the experiment conducted by Davidson (1980a), the diets which contained untreated and micronized peas were critically low in methionine compared to the control diet (1.9 vs 3.1 g/kg). Methionine is the first limiting amino acid in peas and since micronization increased bioavailable methionine as well as that of cystine (Igbasan and Guenter, 1996d), this might put the diet that contained micronized peas at advantage over the diet with untreated peas. This disparity in results may in part, be related to variation in response to heat treatment which may be dependent on the forms and conditions of heat treatment, the concentrations of antinutritive factors in peas and the differences in nutrient density of diets used in both studies. Similar variable response to heating has also been noted for faba beans (*Vicia faba*) (Davidson 1980a).

***Experiment 12***

Cultivar influenced ($P \leq 0.05$) egg production, daily feed intake, egg weight, egg mass output, egg yolk colour, shell thickness but not feed conversion, albumen quality or body weight (Table 54). The effect of experimental period was significant ($P \leq 0.05$) for egg production, egg mass output, shell thickness and egg weight. These performance traits followed the trend described for experiment 11. No effect of enzyme supplementation, cultivar by enzyme, cultivar by period, enzyme by period and cultivar by enzyme by period interactions were observed on all performance traits.

TABLE 54. Degrees of significance, *F* test, of main effects and interactions of cultivar (C), enzyme (E) and experimental period (P) on performance traits of laying hens fed three types of peas supplemented with three levels of enzyme, Experiment 12.

| Source of variation | C | E | P | C x E | C x P | E x P | C x E x P |
|---|---|---|---|---|---|---|---|
| Rate of lay, % | * | NS | ** | NS | NS | NS | NS |
| Daily feed intake, g/bird | * | NS | NS | NS | NS | NS | NS |
| Feed conversion, kg/doz. | NS | NS | NS | NS | NS | NS | NS |
| Egg weight, g | * | NS | *** | NS | NS | NS | NS |
| Egg mass output[1], g/h/d | * | NS | ** | NS | NS | NS | NS |
| Yolk colour score | *** | NS | NS | NS | NS | NS | NS |
| Albumen height, mm | NS | NS | NS | NS | NS | NS | NS |
| Shell thickness, mm x $10^{-2}$ | ** | NS | ** | NS | NS | NS | NS |
| BWC, kg | NS | NS | NS | NS | NA | NA | NA |

* Effect significant at $P \leq 0.05$.
** Effect significant at $P \leq 0.01$.
*** Effect significant at $P \leq 0.001$.
NS = Not significant; BWC = Body weight change; NA = Not applicable.

Productivity of laying hens as influenced by cultivar and enzyme supplementation is presented in Table 55. At the same inclusion rate (650 g/kg), layers fed Impala peas (yellow-seeded) produced more ($P \leq 0.05$) eggs, had higher ($P \leq 0.05$) egg mass output and higher ($P \leq 0.05$) daily feed intake than those fed Radley (green-seeded) and Sirius (brown-seeded) peas. Feed conversion, albumen quality and body weight were the same among peas. Egg yolk colours were darker ($P \leq 0.05$) and egg shells were thicker ($P \leq 0.05$) from layers fed Radley peas. Egg weight was slighlty higher ($P \leq 0.05$) from layers fed Sirius peas. Only two deaths were recorded for the entire flock over the 8-wk period and there was no evidence that treatments had influence on mortality.

The performance of laying hens was quite variable among cultivars. The variability may be related to differences in nutrient composition and availability as reported earlier (Igbasan and Guenter 1996a). Significant differences were found between these cultivars for $AME_n$, starch, and apparent protein digestibilities. Protein digestibility was higher in Impala than in Radley and Sirius. Also, Sirius peas is reltively high in tannin content while Impala and Radley peas contained negligible amounts. The differences in performance became apparent at 650 g/kg inclusion rate in laying diets as against 400 g/kg in broiler diets (Igbasan and Guenter 1996a). This is because broilers are more responsive to antinutrient factors and to small changes in nutrient composition than laying hens.

Supplementation of pea-based diets with pectinase enzyme at the level (50 U/kg) recommended by the manufacturer did not produce any significant effect on egg production, daily feed intake, feed conversion, egg weight, egg mass output, egg yolk

TABLE 55. Performance of laying hens fed three types of peas supplemented with three levels of enzyme, Experiment 12.

| Performance trait | Cultivar | | | | Enzyme | | | |
|---|---|---|---|---|---|---|---|---|
| | Impala | Radley | Sirius | SEM | 0 u/kg[1] | 50 u/kg[1] | 100 u/kg[1] | SEM |
| Rate of Lay, % | 84.8a | 81.7b | 82.0b | 1.01 | 81.9a | 83.6a | 83.0a | 1.01 |
| Daily feed intake, g/bird | 111.3a | 107.6b | 108.8b | 0.71 | 109.3a | 109.9a | 109.4a | 0.71 |
| Feed conversion, kg/doz. | 1.59a | 1.58a | 1.60a | 0.018 | 1.61a | 1.59a | 1.59a | 0.018 |
| Egg weight, g | 61.0b | 60.2b | 61.8a | 0.46 | 61.0a | 61.2a | 60.7a | 0.46 |
| Egg mass output[2], g/h/d | 51.6a | 49.3b | 50.6b | 0.61 | 50.0a | 51.2a | 50.3a | 0.61 |
| Yolk colour score[3] | 5.3c | 6.5a | 5.6b | 0.06 | 5.7a | 5.7a | 5.8a | 0.06 |
| Albumen height, mm | 8.7a | 8.6a | 8.6a | 0.09 | 8.6a | 8.7a | 8.6a | 0.09 |
| Shell thickness, mm x $10^{-2}$ | 34.7c | 36.5a | 35.5b | 0.26 | 35.7a | 35.7a | 35.1a | 0.26 |
| BWC, kg | 0.06a | 0.04a | 0.05a | 0.007 | 0.05a | 0.04a | 0.05a | 0.007 |

[1] Inclusion level of enzyme in the diet.
[2, 3] Same as Table 53.
abc Means within a row under cultivar or enzyme followed by different letters differ significantly ($P \leq 0.05$).

colour, albumen quality, shell thickness and body weight. Increasing the inclusion level (100 U/kg) of pectinase enzyme in the diets did not produce any response in production performance.

Enzyme supplementation of laying hen diets has not been extensively studied. Few studies (Brufau *et al.* 1994; Aimonen and Nasi 1991; Al Bustany and Elwinger 1988) reported in the literature concentrated on cereal grains (wheat, barley, oats, and rye). None of these studies showed any significant improvement in laying performance by supplementing diets with crude enzyme preparations. Our recent experience with broiler chicks (Igbasan and Guenter 1996a) also revealed that addition of pectinase enzyme to pea-based diets did not affect feed conversion which agrees with the current study. However, feed consumption of these birds was improved which also resulted in an improvement in weight gain. Lack of significant response of broiler chicks and laying hens to pea-based diets supplemented with exogenous enzymes suggests that the antinutritive effects of nonstarch polysaccharides in peas may not be of serious concern when compared with those in cereal grains (β-glucans and pentosans), which can create a very viscous intestinal fluid that results in a poor nutrient utilization and wet litter conditions (Campbell *et al.* 1986).

The results of this study demonstrate that untreated peas (irrespective of cultivars) decreased egg production, feed conversion and egg mass output but improved egg yolk colour when included in laying hen diets at 600 g/kg. Dehulling could not reverse the negative effects encountered by feeding high amounts of peas to laying hens but micronization eliminated these effects. The use of pectinase enzyme in pea-based diets

did not improve laying performance under the conditions of this study.

# CHAPTER SIX

## GENERAL DISCUSSION

The use of peas as protein and energy supplements in poultry diets is limited in part due to the lack of adequate nutritional information. In the present research, the nutrient composition, the feeding value, and the potential for improving the nutritive quality of peas by processing and enzyme supplementation were investigated.

Twelve cultivars of peas comprised of 8 yellow-seeded, 2 green-seeded and 2 brown-seeded peas which are widely grown in Western Canada were compared for differences in chemical composition. The cultivars were analyzed for protein, amino acid, starch, dietary fibre and tannin contents. A broad range in protein contents (207.5-264.0 g/kg) with a mean value of 235.0 g/kg was identified. The concentrations of several AAs also varied among the cultivars. The cultivars were high in lysine contents but low in methionine and cystine contents, a characteristic of grain legumes. Although the number of green- and brown-seeded peas included in this study is small, there was no evidence to suggest that these variations are related to seed coat colour. The variation in nutrient contents could be a reflection of the conditions under which the cultivars were grown (Ali-Khan and Youngs, 1973) or inherent varietal differences as reported by Matthews and Arthur (1985). In general, the contents of protein and AAs reported in this study are similar to those reported previously (Marquardt and Bell, 1988; Savage and Deo, 1989).

Relative to the nutritional needs of poultry, peas could be regarded as a moderate source of protein, adequate in lysine but deficient in methionine and cystine. When used in mixed diets especially with cereal grains, which are deficient in lysine but rich in methionine and cystine (NRC, 1994), pea protein and proteins from cereals are complementary, enhancing each one's value.

The pea cultivars were found to contain relatively high starch contents (385.3-436.8 g/kg) which are similar to those reported by Gatel and Grosjean (1990). Since starch is the main dietary source of energy, peas could be used as an energy soure in poultry feeding. The contents of dietary fibre were slightly higher in the brown-seeded cultivars than in the yellow- and green-seeded pea cultivars. Although the non-starch polysaccharides constituted the major components of the dietary fibre, other components included cell wall protein, ash and lignin with associated polyphenols. The lignin with associated polyphenols were higher in the brown-seeded cultivars, which may explain the relatively high content of dietary fibre in these cultivars. A weak negative relationship ($r = -0.46$) between dietary fibre and protein content found in this study may suggest that the dilution effect of dietary fibre on protein and overall nutritive value of peas may not be significant. The brown-seeded cultivars were high in tannins while the yellow- and green-seeded cultivars contained negligible amounts. High tannin contents have also been reported by Griffiths (1981) for dark-flowered pea varieties. The tannins were confined to the seed coat.

Based on the wide variations in the protein and AA contents observed amongst the 12 cultivars evaluated for chemical composition, a study was initiated to evaluate the contribution of location, N application and *Rhizobium* seed inoculation to variations in seed protein content and AA composition of field peas. Eight levels (56, 75, 100, 125, 150, 200, 250, and 300 kg/ha) of N application were tested in 2 different locations. Seed protein contents varied from 248 to 266 g/kg and from 223 to 258 g/kg for the 2 locations. With each increment in N fertilization, protein content increased linearly which suggests that the protein content of field peas is a function of the N status of the soil. Seed inoculation with N-fixing bacteria, however, had no effect on seed protein content. Since AA composition changes according to protein level, percent AAs in DM increased with increasing levels of N application, however, on a protein basis, the concentrations of most essential AA decreased with increasing levels of N application. The only exception was arginine which strongly increased with increasing levels of N application, suggesting that the arginine content of peas is influenced by environment and N application while other essential AAs are influenced more by genotype than environment and N application. This observation was also reported by Kalloo (1993). Therefore the variations in protein content and AA composition observed amongst the 12 cultivars studied could be attributed in part to differences in location and fertilizer application. Our findings were in agreement with previous reports by Eppendorfer and Bille (1974) and Andersen *et al.* (1983).

The nutritive quality of any feedstuff does not only depend on its nutrient composition but also on digestibility of these nutrients by animals. Therefore, the nutrient digestibility of these 12 cultivars was determined using adult cockerels. The TME values ranged from 11.6 to 13.3 MJ/kg while the $TME_n$ values ranged from 11.0 to 12.9 MJ/kg. Nitrogen correction resulted in a 3.4-5.3% reduction in the TME values of peas. The lowest metabolizable energy value was obtained from the cultivar (Sirius) that contained some tannins. The $TME_n$ contents are within the range (10.09 -12.83 MJ/kg) reported by Sibbald (1986). In comparisons with energy feedsuffs (NRC, 1994), the energy value of peas is comparable to that of barley but lower than those of wheat and corn. The mean availabilities of different AAs ranged from 75.9 to 89.6% with TSAA having the lowest value and glutamic acid having the highest value. The yellow- and green-seeded cultivars had a higher AA availability than the brown-seeded cultivars. The lower values in AA availability in brown-seeded cultivars may be due to the presence of tannins. Tannins reduce the digestibility of protein and AAs as a result of the formation of insoluble enzyme-resistant complexes with tannins (Marquardt, 1989; Jansman, 1993). The mean availability value for individual AAs was comparable to that of raw soybeans (Heartland lysine, 1995) and canola meals (Simbaya, 1995).

Considerable work has been carried out to determine the inclusion levels of raw peas in broiler and laying hen diets although data published from these works are contradictory and inconclusive. To establish the replacement value of peas in broiler and

laying hen diets, 3 cultivars, Impala, Radley, and Sirius, representing yellow-, green- and brown-seeded peas respectively, were selected from the 12 cultivars evaluated for chemical composition. The $AME_n$ and starch digestibility in broiler chicks were similar for Impala and Radley cultivars but lower for the Sirius cultivar. Significant differences were found between all cultivars in protein digestibility (APD). The APD value was lower in Sirius than in Impala and Radley cultivars. Judging from the chemical compositions of these 3 cultivars, a possible explanation for the lower nutrient availabilities in the Sirius cultivar might be the presence of tannins. The $AME_n$, APD and starch digestibility values obtained in this study were in good agreement with the values obtain by Conan and Carre (1989) and Brenes *et al.* (1993).

When each of these cultivars were included in broiler diets at 100, 200 and 400 g/kg replacing wheat and soybean, weight gain and feed conversion of broilers fed diets containing 100 or 200 g/kg of any of the pea cultivars were not different from those birds fed the wheat-soybean control diet. The only exception was the poorer feed conversion of birds fed the diet containing 200 g/kg of the high tannin Sirius peas. Regardless of cultivar, inclusion of peas in broiler diets at 400 g/kg depressed overall performance. However, when an excess of CP and EAAs (115% of NRC, 1994) were provided in the diets which contained 400 g/kg peas, weight gain and feed conversion were similar to the wheat-soybean control diet. This suggests that it is possible to alleviate some of the growth depressing effects of the antinutritive factors present in peas by providing

supplementary protein and EAAs in excess of NRC (1994) requirements. Probably the excess CP and EAAs provided in the diets were used to produce more trypsin, a proteolytic enzyme that is rich in AAs especially S-containing AAs (Kempen, 1993) or as tannin binding agents. This action would diminish or eliminate the harmful effects of trypsin inhibitors and tannins resulting in an improvement in the performance of broiler chicks. However, supplementing pea diets with methionine alone in excess of the NRC (1994) recommendation for broiler chicks did not produce significant responses in weight gain and feed conversion. The present results are in agreement with those of Moran *et al.* (1968) who found significant depression in performance of growing chicks fed a diet containing 350 g peas/kg diet but are in contrast to those of Brenes *et al.* (1989 and 1993) who showed that inclusion of peas into chick diets up to 800 g/kg had no detrimental effects on growth performance. However, these authors did not indicate if the good performance observed in their studies was due to the high level (105 g/kg) of sunflower oil in the pea diets which can result in greater palatability. The differences in diet compositions coupled with variations in nutrient and antinutrient contents in peas may be responsible for some of the inconsistencies among reports in the literature.

Layers fed diets containing the 3 cultivars at 200 g/kg diet performed better than their counterparts fed the wheat-soybean control diet. This could probably be due to the fact that at this level of inclusion a better balanced nutrient profile was achieved. With 400 g peas/kg diet, performance of laying hens was similar to the control diet.

Irrespective of cultivar, inclusion of peas into laying hen diets at 600 g/kg decreased production performance. This decrease in performance could also be due to the accumulation of toxicants at this level of inclusion. There was a progressive improvement in egg yolk colours as the level of peas in the diet increased, reflecting the quantity of xanthophylls present in peas. This observation suggests that apart from using peas as protein and energy supplements, peas can also serve as a source of xanthophylls in the wheat/barley:soyabean meal diets thereby reducing the cost of supplementing laying hen diets with synthetic xanthophylls. Also, where consumers have preference for darker egg yolk colours, using peas in laying hen diets may have another economic benefit. The response of egg shell quality (determined as shell thickness and specific gravity) to the level of peas in laying hen diets was not consistent. Shell quality decreased with increasing level of yellow or brown peas in the diets whereas with green peas it was not affected. Ivusic *et al.* (1994) have reported a decrease in shell quality of laying hens fed 590 g/kg yellow peas. There was no obvious reason associated with this poor shell quality observed in both studies. These results indicate that layers fed high amount of peas may require higher calcium supplementation in their diets. Supplementation of laying hen diets containing moderate quantity of peas (400 g/kg) with methionine to either 15 or 30% above NRC (1994) requirement for laying hens had no effect on all production parameters. In general, these results are in agreement with those of Moran *et al.* (1968) and Anderson (1979) but are in contrast to those of Castanon and Perez-Lanzac (1990)

and Ivusic *et al.* (1994). Castanon and Perez-Lanzac (1990) included up to 500 g/kg peas into layer diets without any negative effect on egg production while Ivusic *et al.* (1994) fed up to 590 g/kg peas to layers and observed no effect on performance (except for shell quality). These inconsistencies in results may in part be attributed to differences in the quality of peas and the composition of diets used in different studies. Ivusic *et al.* (1994) used a yellow pea variety *Miranda* in their studies. Savage *et al.* (1986) fed up to 500 g/kg of the same pea variety to turkey broilers from 0 to 16 wk of age without any adverse affect on growth rate, feed utilization and carcass quality. It is possible that this particular variety is low in antinutrient factors.

The presence of toxic substances in peas and lack of accessibility of nutrients to enzyme hydrolysis are often implicated for poor digestibility of nutrients and subsequent poor performance of birds fed diets containing peas (Moran *et al.* 1968; Longstaff and McNab, 1987). In this study, an attempt was made to improve the nutritive quality of peas through processing techniques such as dehulling, micronization (at a temperature between 110 and 115 C for 55 s) and enzyme supplementation. Dehulling increased protein content and the concentrations of most AAs, an effect which was attributed to the removal of the dilution effect of fibre. On average, the NDF content across the 3 cultivars was decreased by 30.5%. On the other hand, micronization had little effect on the chemical composition of peas. Regardless of cultivar, micronization improved metabolizable energy ($TME_n$ and $AME_n$) values of peas in adult cockerels and broiler

chicks, respectively. The availabilities of most AAs were significantly improved by micronization, however, the availability of lysine was decreased. Heat damage of protein at high temperatures has long been recognized. This results from the formation of Maillard reaction products, which are condensation and polymerization products of free amino groups of amino acids such as lysine or histidine with sugar aldehyde groups (Bjarnason and Carpenter, 1970). Maillard reaction products are not digestible and in turn, unavailable to the animals. Micronization also increased the APD and starch digestibility values of peas in broiler chicks. On the other hand, dehulling only improved the metabolizable energy content ($AME_n$) and APD value of the brown-seeded pea cultivar (Sirius). This could be due to the fact that the hull fraction of this cultivar contained an appreciable quantity of tannins. Brenes *et al.* (1993) had shown that dehulling a tannin-containing Maple pea cultivar improved its nutritive value. The improvements in the digestibility of peas as a result of micronization suggests that some form of heat treatment is required to inactivate the antinutritive substances present in peas and to disrupt the cell wall structure of peas to facilitate nutrient-enzyme interaction. Zuilichen and Poel (1989) found that micronization of peas at 124 C for 65 s completely destroyed trypsin inhibitor activity whereas Longstaff and McNab (1987) and Carre *et al.* (1991) also demonstrated that autoclaving and steam pelleting improved energy, protein and starch digestibility of peas in adult cockerels and broiler chicks.

When feeding micronized peas to broiler chicks and laying hens, the performance

of these birds were significantly improved. Broiler chicks fed micronized peas grew faster and had better feed conversion than their counterparts fed the untreated peas. The performance of laying hens also followed the same trend. Further improvements in chick performance was also observed when micronized peas were supplemented with L-lysine which suggests that the biological availability of lysine in micronized peas may be a concern. The improvements in nutrient utilization of micronized peas resulted in improvements in performance observed both in broiler chicks and laying hens. In agreement with the current findings, Davidson (1980b) reported improvements in egg production of laying hens fed micronized peas. Dehulling was more beneficial when applied to brown-seeded pea cultivar which contained tannins. Also, only broiler chicks showed positive response to dehulled peas whereas, laying hens did not respond because the tannin content of this cultivar is not high enough to influence production performance as shown in part of the work reported here. These findings confirmed previous reports that dehulling, when applied to tannin-containing faba beans (Marquardt and Ward, 1979) and peas (Brenes *et al.*, 1993) improved the performance of chicks.

Because pectic substances form substantial amounts of the polysaccharides found in peas (26-55% of cell wall material from pea cotyledons and 16.8% from hulls) (Reichert, 1981; Brillouet and Carre, 1983), the effects of supplementing pea-based diets with pectinase on the performance of broiler chicks and laying hens were investigated. Supplementing pea diets with pectinase improved weight gain and feed consumption in

broiler chicks. However, there was no effect on feed conversion efficiency. Since feed conversion was not affected, the improvement in feed consumption could be attributed to an increase in intestinal passage rate which stimulated appetite. The improvement in feed consumption resulted in an overall improvement in weight gain. Supplementing pea diets with pectinase had no positive effects on the production performance of laying hens. Brenes *et al.* (1993) supplemented pea diets with cellulase, amylase and protease preparations and found no response in chick performance. The lack of response of broiler chicks and laying hens to pea diets supplemented with exogenous enzyme suggests that the effects of non-starch polysaccharides in peas may not be of serious concern when compared with those of barley, wheat, rye, oats, and triticale. These cereral grains contain structural carbohydrates such as arabinoxylans and β-glucans that can form viscous materials which can act as barriers to diffusion of nutrients within the gut lumen, reducing enzyme-substrate contacts and limiting rate and amount of nutrient absorption (Campbell and Bedfrod, 1992; Guenter, 1993).

A large proportion of pea seeds produced are processed into different fractions like pea hulls, pea starch and pea protein concentrates to enhance their utilization in the food industry. This processing results in unavoidable losses of portions of the pea flours. Among these products are "pea chips", which according to the Process Manager, Woodstone Foods Corporation constitute industrial wastes because there is no market for them. This research was extended to cover the feeding value of pea chips in broiler diets.

The $AME_n$ and APD values obtained for pea chips were slightly higher than those obtained for any of the three cultivars. This may be attributed to the partial removal of hulls from the pea chips which reduced the fibre contents. When pea chips constituted 150, 300 and 450 g/kg of broiler diets, weight gain and feed conversion were lower for birds fed diets containing 300 or 450 g/kg but the performance of birds fed diets containing 150 g/kg pea chips were not affected. This further confirmed that inclusion of high amounts of raw peas in broiler diets decreased growth rate and feed utilization.

# CHAPTER SEVEN

## SUMMARY AND CONCLUSIONS

Detailed chemical analyses were performed on 12 cultivars of peas. Also, the digestibility of these peas in young and adult cockerels was determined. On the basis of the chemical composition and nutrient digestibility, 3 cultivars were selected for feeding trials with broilers and laying hens. They were included in diets at various levels. The potential for improving the nutritive value of peas in poultry diets through micronization, dehulling and enzyme supplementation was also studied. The following conclusions can be drawn from this research.

1. The nutrient composition of peas vary widely and part of these variations can be attributed to location and N fertilization. Therefore, in order to formulate balanced diets containing peas, regular analysis may be required to determine the nutrient contents which are of concern in poultry nutrition.
2. Peas are relatively high in ME values and as a result they can replace a significant proportion of the dietary energy commonly supplied by conventional grains. Amino acid digestibility values of peas are also comparable to those of conventional protein supplements, raw soybean meal and canola meal. Peas are nutritionally deficient in the sulphur amino acids of methionine and cystine.
3. The brown-seeded cultivars used in this study contained tannins and these seemed to have detrimental effects on their nutritive quality.

4. For optimum performance, the inclusion level of raw peas should be restricted to 200 g/kg in broiler diets and 400 g/kg in laying hen diets. Adequate methionine supplementation of diets containing peas is necessary.
5. Satisfactory performance of broiler chicks could be maintained at 400 g peas/kg diet provided crude protein and essential amino acids are supplied in excess (15% above) of the NRC requirements.
6. Egg yolk colours consistently improved as the level of peas in laying hen diets increased.
7. The response of egg shell quality to varying levels of peas in laying hen diets was not consistent.
8. Application of micronization (infrared heat treatment) improved the digestibility of peas and the performance of birds fed diets containing micronized peas.
9. Micronization decreased the availability of lysine, however, this adverse effect was alleviated by supplementing diets containing micronized peas with crystalline L-lysine.
10. Dehulling was more beneficial when applied to the pea cultivar which contained an appreciable quantity of tannins located in the hulls.
11. Supplementing pea-based diets with pectinase enzyme improved weight gain and feed consumption but not feed conversion of broiler chicks. Laying hens did not respond to pectinase supplementation.
12. Pea chips can be used in broiler chick diets; the inclusion rate is similar to that of whole peas.

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