HGCA

PROJECT REPORT No. 170

TRUE DIGESTIBILITY OF WHEAT PROTEIN AND AMINO ACIDS IN BROILERS

SEPTEMBER 1998

Price £8.00

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TRUE DIGESTIBILITY OF WHEAT PROTEIN AND AMINO ACIDS IN BROILERS

by

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This is the final report of a three year project which commenced in October 1992. The work was funded by a grant of £181,876 from HGCA (project no 0016/2/92).

CONTENTS

Section A

SUMMARY

Wheat Proteins

1 Introduction 1

2. Protein content of wheat 1
2.1. Cytoplasmic or metabolizable protein 2
2.1.1. Enzymes and inhibitors 3
2.1.2. Purothionin 3
2.2. Storage proteins 3
2.2.1. Gliadins 4
2.2.1.1. Quality 4
2.2.1.2. Structure 5
2.2.1.3. Amino acid composition of gliadin 5
2.2.2. Glutenins 6
2.2.2.1. Structure 7
2.2.2.2. Amino acid composition of glutenins . . . 8

3. Protein accumulation 8
3.1. Protein in yield 10
3.1.1. Protein accumulation in the field 10
3.2. Nitrogen content 10
3.2.1. Effect of the environment 11
3.3 Storage proteins 12
3.4. Starch/protein associations 13

4. Nutritive value of wheat proteins 14
4.1 Requirements of poultry 15
4.2 Amino acid interactions and maintenance requirements . . . 16
4.2.1. Amino acid interactions 17
4.2.1.1. Lysine and arginine 17
4.2.1.2. Leucine, valine and isoleucine 17
4.3. Digestibility 18
4.3.1. Digestion of proteins in poultry 18
4.3.2. Digestibility of amino acids 19
4.3.3. Digestion experiments 20
4.4.1. True and apparent digestibility 20
4.4. Other methods used to assess protein quality 22

Section B

Experimental Programme

1. Introduction 24

2. Experimental work 25
   2.1. Trials conducted 26
      2.1.1. Trial 1 26
      2.1.2. Trial 2 27
      2.1.3. Trial 3 27
      2.1.4. Trial 4 27
      2.1.5. Trial 5 28
      2.1.6. Trial 6 28
   2.2. Chemical analyses 29
   2.3. Determination of the coefficient of amino acid digestibility 30
   2.4. Apparent digestible amino acid content of diets and of wheats 30

3. Results and discussion 33
   3.1. Trials 35
      3.1.1. Trial 1 35
      3.1.2. Trial 2 35
      3.1.3. Trial 3 36
      3.1.4. Trial 4 37
      3.1.5. Trial 5 37
      3.1.6. Trial 6 38
   3.2. Nitrogen content and content of digestible amino acid 45
   3.3. Coefficient of apparent amino acid digestibility and nitrogen content 50

4. References 56

5. Appendices 65

SUMMARY

Wheat is a major constituent of poultry diets, accounting for up to 0.8 of the metabolizable energy and 0.4 of the protein requirement of broilers. Variation in protein quantity and quality, due to environmental conditions and variety, may influence performance of broilers. Increasingly, digestible rather than total amino acids are being used as bases for diet formulation. However procedures involved in evaluating digestibility often need to distinguish between true and apparent digestibility, necessitating assessments of endogenous losses.

The objective of this study was to assess the digestibility of amino acids from specific wheat varieties, employing a technique based upon the use of an inert dietary marker and ileal sampling of digesta. Three diets per wheat sample were formulated containing 250, 500 and 750 g/kg wheat. The other constant dietary constituents were (per kg) oil (50 g), mineral/vitamin mix (50 g) and 5 g marker (titanium dioxide). Diets were made up to 1000 g/kg with a 50:50 starch/glucose mix. Diets were fed *ad libitum* for 3 days to 6 cages of 2 male Ross broiler chickens of initial age 18 days, housed in an environmentally-controlled room. Birds had constant access to water.

After 3 days on treatment, samples of ileal digesta were obtained from the birds and analysed for amino acid and titanium dioxide content.

During the study a method for the determination of titanium dioxide, the inert dietary marker, was developed for use with samples of 0.1g. The method relied on the colour change between titanium dioxide and hydrogen peroxide

The apparent digestibility of each amino acid was calculated by regressing digestible amino acid content in the wheat sample on wheat inclusion rate and extrapolating to 1000g wheat / diet. Regression relationships were usually highly significant. True digestibility was calculated by deducting the

ordinate abscissa term.

Six trials in total were carried out. The first 3 used named varieties :

Trial 1 used the wheat variety Mercia which had been treated with 3 levels of nitrogen fertilizer.

Trial 2 also used the variety Mercia.

Trial 3 used two varieties Brigadier and Hussar at 2 crude protein (CP) levels: Brigadier 102, 130 and Hussar 96, 128 g CP/kg.

It was then thought more appropriate to investigate wheat samples with a similar genetic background and minimal genetic differences (*ie* near-isogenic lines). This was because two varieties may be distantly or closely related and the name alone of the varieties would give no indication of closeness of genetic background. Furthermore, even within a variety, two separate samples could show considerable genetic diversity. Using near-isogenic lines allows differences to be related to the presence of specific genetically defined characteristics against an otherwise comparatively uniform genetic background.

Trial 4 examined two wheat samples, which were isogenic except for endosperm hardness and protein, and their parent varieties Hobbit /Avalon 5A

Trial 5 investigated the differences in digestibility in two of the first varieties to be used in the genetical modification of wheat.

Trial 6 evaluated the effects of the introduction of a rye gene (1B/1R) on digestibility.

Results revealed that:

- Significant variation in amino acid digestibilities existed between wheats in individual trials.
- Apparent digestibility values for individual amino acids within wheats were significantly different.
- Results suggested that the use of a constant conversion factor to estimate amino acid digestibilities for a particular wheat is invalid.
- There appeared to be variation in the differences between true and apparent digestibility.
- Increasing nitrogen fertilizer levels tended to increase the digestibility, as did an increase in crude protein content.
- The inclusion of the Avalon 5A substitution (thought to affect the protein characteristics) to the Hobbit line appeared to decrease digestibility.
- Insertion of the rye 1B/1R gene appeared to decrease digestibility.
- For certain amino acids, as the nitrogen content of the grain increased, the coefficient of apparent digestibility of that particular amino acid increased.
- The data presented have considerable implications for the animal feed industry, plant breeders and, ultimately, growers.

1. INTRODUCTION

In the UK wheat is the main ingredient used in poultry diets. This is mainly as a result of its high starch content and because it contains protein. Thus up to 0.8 of total dietary energy for broilers and 0.4 of the total protein requirement may be derived from wheat.

The nutritional value of feeds such as wheat is determined by digestibility of starch (which will influence metabolisable energy values) and protein / amino acids in the grain. Digestibility is generally recognised as the basis for evaluating the wheat quality to the bird and increases the accuracy of diet formulation.

2. PROTEIN CONTENT OF WHEAT

Generally the protein content of wheat lies between 60 and 200g /kg. Variation is due to different environmental conditions prevailing during growth and has also been found to be a weakly inherited character. Breeding of wheats has resulted in increasing yields, but often with a corresponding decrease in protein content (Kapoor and Heiner, 1976; Kingston, 1980).

It has generally been accepted that protein is unevenly distributed within the grain with a proportion of 0.70 - 0.75 in the endosperm, 0.19 in the aleurone layer and 0.08 in the embryo (e.g. Kingston, 1981)

Initial analysis of wheat proteins, based upon their extractability characteristics, led them to be divided into groups: albumins, globulins, gliadins and glutenins (Osborne, 1907).

However, protein components of wheat are notoriously difficult to separate and extraction is dependent upon several factors:

1. Temperature

2. pH
3. Duration of extraction
4. Solid : solvent ratio
5. Association of protein with lipid and carbohydrate.

Graveland *et al.,* (1979) suggested the following reasons for the extraction difficulties:

1. Proteins are in a complex structure along with starch, lipids, and pentosans forming a compact flour particle requiring careful and detailed extraction methods.
2. The majority of wheat proteins are insoluble in water or salt solutions.
3. Wheat proteins strongly associate through hydrogen bonding, ionic reactants and participate in hydrophobic bonding.

Proteins in seeds have two main functions which are described below.

2.1. Cytoplasmic or metabolically active protein

These include water, salt or buffer soluble (Preston and Kruger, 1976). Examples are albumins and globulins (predominantly in the embryo).

Unlike the storage proteins, these have not been widely studied and have been found to have very little homogeneity. The group includes enzymes, enzyme inhibitors and purothionin.

2.1.1. Enzymes and inhibitors

Little is known about these enzyme proteins, mostly because of the small quantities found. Enzymes such as amylases and proteinases are present which,

in the latter case, can be involved in the Maillard reaction.

2.1.2. Purothionin

This is a low molecular weight protein which has been extracted from wheat. It was determined to be high in the proportions of sulphur containing amino acids and lysine (proportion of each 0.2). The function of purothionin is unknown and perhaps associates with the endoplasmic reticulum (Carbonero *et al.*, 1980).

AMINO ACID COMPOSITION OF SOME WATER-SOLUBLE PROTEINS OF WHEAT (MOL/10^5g PROTEIN)

Amino acid	Albumin
Lysine	46
Histidine	00
Arginine	52
Threonine	22
Serine	59
Aspartic acid	70
Glutamic acid	99
Glycine	76
Alanine	72
Valine	104
Leucine	70
Isoleucine	16
Proline	69
Tyrosine	31
Phenylalanine	01
Tryptophan	28
Cystine	74
Methionine	24

2.2. Storage proteins

These include gliadins and glutenins and provide a nutrient source for the developing plant.

2.2.1 Gliadins

These proteins have been shown to be very complex and variable between varieties although most, if not all, gliadins are very high in glutamic acid. Glutamine provides the germinating seed with a readily available source of nitrogen. Generally, apart from having very high glutamic acid, aspartic acid and proline levels, the amino acid levels are very low, especially lysine (Lasztity, 1984). Histidine and arginine are also present in low levels as are free carboxyl groups, thus conferring little charge. Some workers have divided gliadins into alpha, beta, gamma and omega groups based upon their electrophoretic mobility (Woychik *et al.,* 1961). Those of the omega group have the highest molecular weight.

2.2.1.1. Quality

Protein quality is usually defined in terms of amino acid content, i.e. their presence and levels. Wrigley *et al.* (1980) compared sulphur rich and sulphur poor endosperm proteins, and discovered that sulphur levels were associated with, and significantly altered, protein quality. Decreasing sulphur levels were associated with altered proportions of protein through increasing the amount of low mobility proteins at the expense of the high mobility ones, i.e. the proportion of polypeptides with a molecular weight less than 8k Daltons increased and those over 28k Daltons decreased. Thus grain with a low sulphur content showed a decreased amino acid content with the exception of arginine and aspartate. This is mainly due to the importance of sulphur in the synthesis of other amino acids, especially cystine, and its role in the formation of disulphide bonds. Cystine and methionine were observed to decrease by 50%, thus they became nutritionally limiting.

There is also evidence of grain quality decreasing after maturity mainly

due to the environment. Environmental factors such as light, moisture, and temperature variations can seriously decrease protein quality. This may result from early germination which causes an alteration in protein metabolism. During germination storage protein is broken down and the protein bodies empty leaving just the membrane, which gives rise to a vacuole (Pernollet, 1978). The structural protein is degraded and new proteins form.

2.2.1.2. Structure

Tatham and Shewry (1985) using spectroscopy, studied the secondary structure of alpha, beta, omega and gamma gliadins. The omega gliadins were devoid of any alpha helix or beta sheet but were rich in beta turns. The other gliadins contained an alpha helix accounting for a third and a beta sheet accounting for a tenth of their structure. They concluded that the omega gliadins are stabilized by strong hydrophobic interactions, whereas the other gliadins are stabilized by covalent disulphide bonds and non-covalent bonds. This structure was also suggested by Lasztity (1984).

2.2.1.3. Amino acid composition of gliadin

Experiments have shown that gliadin fractions are not pure protein. Bushuk *et al.* (1980) reported that approximately a tenth of gliadin is non-protein (of which, proportionally, 0.94 is lipid and 0.06 is carbohydrate). However, the effect of this fraction on protein analysis has not been ascertained.

Amino acid	Anhydro amino acid (g) in 100 g of recovered anhydro amino acids
Lysine	0.64
Histidine	2.23
Arginine	2.72
Aspartic acid	2.86
Threonine	2.16
Serine	4.67
Glutamic acid	38.87
Proline	13.70
Glycine	1.53
Alanine	2.03
Cystine	2.97
Valine	4.12
Methionine	1.43
Isoleucine	4.31
Leucine	6.85
Tyrosine	2.61
Phenylalanine	5.52
Tryptophan	0.71

(Ewart, 1967)

2.2.2. Glutenins

Glutenin appears to have a structure similar to gliadin, but there are significant differences. Glutenin possesses more lysine, glycine and tryptophan, slightly more serine, arginine, tyrosine, threonine and alanine, but less

phenylalanine, isoleucine, cystine and proline. However, values quoted vary tremendously (Ewart, 1968). This is often due to different correction factors or oxidation of amino acids during analysis, especially cystine.

Lasztity (1984) stated that glutenins have a more hydrophilic character than gliadins and suggested that this was due to the difference in amino acid content. It was also evident that there was less variation in the different varieties of wheat, with a slightly increased or decreased basic amino acid content and a slight variation in glutamic acid and proline.

As with the gliadins, the quality can also be seriously affected by the environment and premature germination.

2.2.2.1. Structure

Using SDS PAGE methods it has been found that the protein structure consists of seventeen to twenty polypeptide chains (Orth and Bushuk, 1973 ; Bietz and Wall, 1972). There have been many proposals as to their structure. Ewart (1969) treated glutenin with excess mercaptoethanol and obtained a decreased viscosity by disrupting the disulphide bonds. This confirmed the suggestion that glutenin is made up of glutenin subunits linked head to tail.

A similar model was proposed by Graveland *et al.* (1985) who also questioned the presence of intradisulphide and interdisulphide bonds, and secondary forces holding subunits. It was further suggested that glutenin subunits were linked via interchain disulphide bonds into a linear chain. Tatham *et al.* (1984) suggested that the considerable elastic properties of glutenin were attributable to the presence of repetitive beta turn conformations within the heart of these polypeptides. It was also suggested that alpha-helix rich domains

existed at the nitrogen and carbon termini.

2.2.2.2. Amino acid composition of glutenins

Amino acid	Anhydro amino acid (g) in 100 g of recovered anhydro amino acids
Lysine	2.26
Histidine	2.32
Arginine	4.13
Aspartic acid	3.73
Threonine	3.11
Serine	5.38
Glutamic acid	33.15
Proline	10.29
Glycine	3.82
Alanine	2.77
Cystine	2.37
Valine	4.24
Methionine	1.66
Isoleucine	3.74
Leucine	6.58
Tyrosine	3.61
Phenylalanine	4.76
Tryptophan	2.09

(Ewart, 1967)

3. PROTEIN ACCUMULATION

Two distinct phases of protein accumulation have been observed using in vitro methods. The first occurs during cell division when albumins and globulins are synthesised and the next takes place during the second stage of development when storage proteins become a major product of protein synthesis (Flint *et al.*, 1975; Mecham *et al.*, 1981). Jennings and Morton (1963) also noticed this effect whilst looking at the protein body development

within the wheat endosperm. They observed that the nitrogen content of the grain was apportioned differently as the grain matured, as shown by the following results.

	Storage proteins		**Other proteins**	
Days post anthesis	18	46	18	46
μg storage protein /nitrogen in the grain	81	378	60	110

Thus, the storage proteins increase significantly as a proportion of the total protein. This effect was also suggested by Mecham *et al.* (1981) who observed an increase in gliadin proteins as a proportion of the total protein, as the following results show:-

Days post-anthesis	**Proportion of total protein as gliadin**
6	0.06
9	0.05
12	0.08
15	0.09
18	0.19
24	0.21
30	0.25
39	0.24

Radiolabelling studies had already revealed the presence of gliadins 18-40 days post- anthesis (Graham *et al.*, 1963, Graham and Morton, 1963). Mecham *et al.* (1981) thus suggested that all gliadins were present by the twenty fifth day post-anthesis using the above amino acid composition data of wheat

endosperm.

Glutenin accumulation has been examined to establish whether aggregation occurs as a result of grain desiccation (Bushuk and Wrigley, 1972; Khan and Bushuk, 1978). However Weber and Osborne (1969) suggested that aggregation of glutenin molecules occurred throughout storage accumulation and their subsequent increase was simply due to an increase in molecular weight.

As the grain matures the proportions of non-essential amino acids increase and the essential amino acids decrease. This is due to the relatively greater increase in the storage protein levels compared with the levels of other proteins, which are composed of predominantly essential amino acids and are present in the embryo.

3.1 Protein in yield

3.1.1. Protein accumulation in the field

The concentration of protein in wheat is an important determinant of its value to the animal. As yields of grain increase, protein levels within the grain decrease. Experiments have also confirmed that the energy cost to the plant is slightly greater for protein than for starch accumulation (CSIRO, 1979). It was also suggested that problems with low protein levels also result in a wide variation in concentrations of the constituent amino acids.

3.2. Nitrogen content

It has been found that protein levels increase over the time of protein body development, although non-protein nitrogen levels decrease (Kapoor and Heiner, 1976). At twelve days the proportion of non-protein nitrogen was 0.33

of N in the grain falling to 0.04 in the mature grain.

3.2.1. Effect of the environment

Mineral deficiencies can seriously affect the development of a plant, especially where protein is concerned. Nitrogen deficiencies result in a decrease in the total protein, and sulphur deficiencies alter protein within and between protein classes. Wrigley *et al.* (1980) observed that gliadins were affected such that those of high molecular weight increased and those of low molecular weight decreased. Glutenins were also affected due primarily to the decrease in sulphur causing a reduction in cystine. Cystine in glutenin is important in the formation of the disulphide bonds which contribute to the structure of the protein by joining the polypeptide chains.

Nitrogen can also alter the amount of a particular protein class which develops. It is generally accepted that as nitrogen levels increase the level of gliadins and glutenins increase considerably, whilst the levels of albumins and globulins only show a slight increase. Grain quality can also be affected significantly by inclement weather, mainly due to sprouting and mould development. Sprouting results in a decrease in both protein and starch content, thus decreasing the nutritive value (CSIRO, 1985). Mould growths often produce toxins especially aflatoxins which can be lethal and almost always decrease productivity. Chicks have been found to be less affected than cattle and pigs (Howell, 1982), although growth is retarded in young chicks and egg production decreases in hens.

Effects of aflatoxin in chicks

BIRD	TOXIN LEVEL (mg per kg feed)	EFFECT
broiler chicks	0.44	none
	0.80	liver changes
	1.60	liver and growth affected
	2.50	affect on liver and growth severe, death
laying hens	0.63	bruising increases
	2.50	body weight decreases

3.3. Storage proteins

The storage proteins in mature cereals are present in the form of small spherical bodies 1-10 micrometers in diameter known as protein bodies. Protein bodies were first demonstrated in 1883 and were then called aleurone grains. Other terms such as aleurone vacuole, protein vacuole, protein grain and protoplast have also been used. Most experiments have been undertaken using microscopic techniques following isolation in aqueous solutions and fixing (Pernollet 1978). However, in older grains the protein bodies are normally compressed so much that they are no longer individual.

Storage proteins generally first appear 10-12 days after flowering and develop to fill the vacuolar space within the cell. Most of these proteins are found in the endosperm either in the cytoplasm or oppressed to a parallel array of lipoprotein membrane. The way in which storage proteins are synthesised and transported to vacuoles has not yet been identified although there are many possible routes. Single or groups of protein bodies are formed initially, increasing in size after fourteen days post-flowering until the twenty second day (Jennings *et al*, 1963; Graham *et al.*, 1963). This has been discovered due to a large increase in acetic acid soluble proteins in grain at this time. Amino acid content differs depending upon the position of protein

in the grain.

Buttrose *et al.* (1963) suggested that storage proteins were synthesised on the rough endoplasmic reticulum (rER) and transported to protein bodies through the Golgi apparatus. Golgi vesicles have been found to contain similar material to that in protein bodies and dictosomes, which have also been observed to be an intermediate part (Buttrose, 1963). However Barlow *et al.* (1974) suggested that Golgi bodies were not involved due to an absence of dictosomes fourteen days after flowering, and that the Golgi bodies alone were unable to carry out the process of synthesis and complete it in the time required.

By 1981 new fixation techniques had been developed (Parker, 1981; Campbell *et al.,* 1981). These revealed that dictosomes were present up to forty days after flowering. Thus there is the possibility that they along with the Golgi bodies are important. Previous results may have been influenced by the poor staining techniques (Parker, 1981). Campbell *et al.,* (1981) and Graham *et al.,* (1963) suggested that initially vacuoles are used to store protein, then as the protein level increases the ER becomes more important and ER distension becomes more noticeable, although it is always involved.

3.4. Starch - protein association

Cell walls of cereals contain many complex carbohydrates and protein bodies have starch granules embedded within their matrix, thus carbohydrates will influence nutrient utilization. McMaster and Bushuk (1983) suggested that specific carbohydrates were associated with such proteins.

Pentosans are the main carbohydrates associated with proteinaceous material especially glutenin and gliadin and are difficult to extract. Wheat

endosperm pentosans are composed of a main chain of betaxylopyranosyl units linked beta-1,4 with L arabinofuranosyl at the 2 or 3 positions of the xylose units .

It has been stated that wheat endosperm contains 150g/kg protein and the rest is carbohydrate of which a proportion of 0.85 are pentosans (Wiseman and Inborr, 1990). Wheat pentosans are of little nutritive value due to their undigestibility and interaction with other nutrients producing complexes, the majority of which are insoluble. Some however are soluble and can increase the viscosity thus reducing the nutrient availability and functionality of the wheat proteins (McMaster and Bushuk, 1983).

In broilers Choct and Annison (1992) observed that the digestibility of wheat nutrients was decreased in the presence of wheat pentosans. By adding wheat pentosans to a standard diet, nitrogen retention, growth, feed utilization and the apparent metabolizable energy (ME) were significantly depressed ($P<0.001$). The depressions were related to the level of pentosan in the diet. The ileal digestibility decreased by 0.187 absolute with levels of arabroxylans of 40 g/kg. Thus, when considering the amino acid digestibility of wheat, the influence of wheat pentosans may be important. Similar previous work by Choct and Annison (1990) had already suggested that pentosans in wheat decrease the food conversion ratio.

4. NUTRITIVE VALUE OF WHEAT PROTEINS

Generally wheat proteins, especially the predominant storage proteins, are poor in the most nutritionally important amino acids. Thus the biological value of wheat is low and it must it be supplemented with other raw materials.

Cytoplasmic proteins in wheat generally have a better amino acid

composition with an increased level of the nutritionally important amino acids including lysine, methionine, and cystine.

Gupta *et al.*, (1976) observed that lysine in wheat protein was too low for adequate human nutrition. This is normally evident once the grain is fully mature due to a decrease in the concentration of lysine as the endosperm develops. This may be because of decreased synthesis of certain proteins or due to preferential degradation of these proteins. Gupta *et al.* (1976) further observed that the synthesis of lysine decreases and degradation increases from eight days post anthesis to twenty eight days.

4.1. Requirements of poultry

Chicks have a definite requirement for protein in the diet to enable growth and metabolism to continue unhindered. The ideal protein source is one containing all of the required amino acids in the correct quantities. Thus the efficiency with which synthesis of proteins occurs depends on the match between the amino acids provided and the amino acids required. In poultry some twenty amino acids are required for protein synthesis, some of which are essential and must be provided in the diet. Others are non essential, and can be synthesised within the chick.

In addition to the need to supply essential amino acids, the diet must contain an optimum balance of them relative to each other. Thus nutritional problems do not just occur in proteins with low amino acid values but also if amino acids are in great excess. It is furthermore preferential that, if diets are to produce the desired results, the amino acid digestibility is known. Most feeds have an estimated protein value based upon the amino acid composition and digestibility.

Essential amino acids
(ARC 1975)

Lysine
Cystine *
Methionine
Threonine
Tryptophan
Isoleucine
Leucine
Valine
Phenylalanine
Histidine
Arginine
Tyrosine +

* can be synthesised from methionine
+ can be synthesised from phenylalanine

Non-essential amino acids

Glycine +
Serine +
Alanine
Aspartic acid
Glutamic acid
Proline +

+ can be synthesised but sometimes not in adequate amounts

Unfortunately not all of the amino acids present in the feed are similarly digestible, with amino acid digestibility being dependent upon (ARC, 1975):

1. physical nature of the feedstuff
2. physiochemical nature of the protein
3. chemical interactions of amino acids, carbohydrates and lipids.

4.2. Amino acid interactions and maintenance requirements

In formulating diets it is generally assumed better to specify a

minimum concentration for crude protein and for the first limiting amino acid, normally lysine. However imbalances themselves may affect the level of amino acid requirements (D'Mello, 1988). It has also been observed that if amino acids are in excess then the animal requires more of the limiting amino acid (Morris *et al.,* 1987).

4.2.1. Amino acid interactions

Different amino acids when present in the feed can interact thus increasing the requirements of one or more amino acid even if it is not in great excess. Amino acids in a small surplus can sometimes increase the need for others. This has been observed during experiments where incomplete mixtures of amino acids have been administered causing depressions in growth (D'Mello and Lewis, 1970).

4.2.1.1 Lysine and arginine

These have for a long time been known to be antagonistic. In one trial as arginine increased growth increased (perhaps it was limiting). However the addition of lysine to the diet caused the growth to decrease progressively. To ensure that growth was unhindered arginine had to be increased. It was also observed that the opposite had the same affect i.e. increasing arginine when lysine levels were low caused a decrease in growth. (D'Mello and Lewis, 1970). However it has become clear that the interaction can be lessened with the addition of electrolytes, for example potassium acetate (O'Dell and Savage, 1966) due to the increase in lysine catabolism or an increase in arginine utilization.

4.2.1.2. Leucine, valine and isoleucine

More recently interactions between these three amino acids have become evident. Increasing leucine concentration increases the valine requirement, increasing the leucine to 14, 24 and 34g per kg required an

increase in the valine content to 7.7, 8.9, and 10.1 respectively per kg diet (D'Mello and Lewis, 1970). It was also demonstrated that increasing leucine also increased the isoleucine requirement. Thus a three way interaction occurs with leucine being responsible for an increase in the requirement for valine and isoleucine (D'Mello, 1988).

4.3. Digestibility

Information on amino acid digestibility is essential in order to evaluate diets and raw materials. To ensure that diets are balanced to provide the optimum amino acid levels, tables are used, based upon chemical analysis of feeds and droppings, assuming that if protein / amino acid is digested then it is available to the animal (Terpstra, 1979). However some amino acids can be digested but not utilized and digestibilities of amino acids are not always equal to the digestibility of the crude protein, especially if the feedstuff is poorly digestible.

4.3.1. Digestion of proteins in poultry

Optimum digestibility of amino acids from protein requires initially a high digestibility in a short a time as possible. This is ensured using a system of endopeptidases and exopeptidases.

Digestion commences in the proventriculus of the fowl. Here inactive pepsinogen is secreted by the chief cells where it is altered to pepsin due to the pepsin already present and an acidic pH. Sturkie (1976) observed that there were actually five pepsinogens.

In the duodenum, jejunum and ileum digestion is completed by pancreatic and intestinal proteases, which are produced as a result of increased secretin, a hormone produced by the gastric mucosa.

In the small intestine an increase in pH also occurs as a result of bicarbonate production from the liver, the gastric acid thus neutralizing the digesta. This creates a suitable pH and environment for the action of the pancreatic enzymes, thus enabling polypeptides to be digested on the brush border luminal membranes of the ileum to peptides. Thus proteins are digested to polypeptides and peptides, and ultimately to amino acids or oligopeptides.

In the ileum the process of absorption commences on the brush border luminal membranes. Most absorption occurs as a result of active transport although many factors influence the absorption of digestion products. These include (Kan, 1975):

1. Active transport of amino acids as polypeptides, against a concentration gradient
2. Competition / interaction between amino acids
3. In vitro studies have indicated that the presence of sugars may result in a decrease in amino acid transfer.

4.3.2. Digestibility of amino acids

Experiments with pigs, poultry and rats have shown that as protein digestion decreases the differences in digestibilities between individual amino acids decreases. However lower digestibility products are cheaper and more readily available thus they are more frequently used in animal feeds and, as previously mentioned, it is essential that protein evaluation is carried out.

The majority of digestibility work involves the feeding of a precise diet and the collection of urine and faeces voided over a set period, accounting for dietary adjustments.

4.3.3. Digestion experiments

Experimental work on digestibility is fraught with difficulty, mainly due to endogenous losses, sampling and collection problems, and microbial fermentation in the gut especially in the caeca. Thus much work has been carried out to estimate values for endogenous losses and the extent of fermentation.

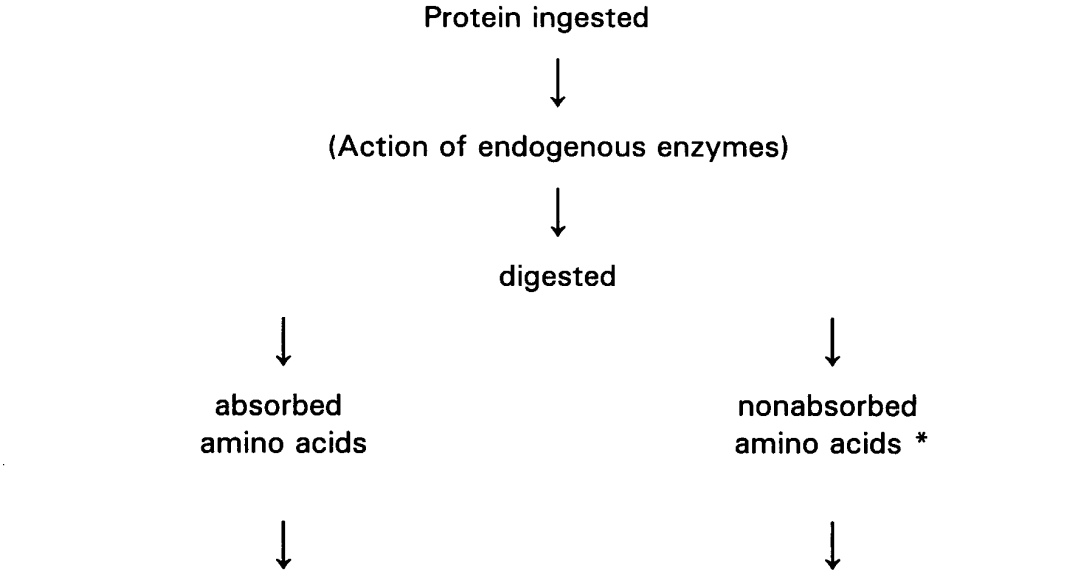
4.3.4. True and apparent digestibility

Methods used to determine amino acid and protein digestibility rely on the principle that the digestibility is equal to the nitrogen eaten minus the nitrogen excreted as a proportion of the nitrogen eaten. This is often quoted as apparent digestion.

However, it is thought by some nutritionists that it would be better to use true digestibility in which endogenous losses can be accounted for, although as previously mentioned some believe that this may be unduly complex. It has been shown that as intake of an amino acid increases, the apparent digestibility becomes almost equal to the true digestibility.

Thus, when a low amino acid diet is consumed, an assessment of endogenous losses must be carried out, although this can be generally ignored. Eggum (1985) stated that the true digestibility coefficient can differ by up to 0.2 absolute from apparent digestibility.

Figure 1 Schematic diagram of digestibility, absorption and metabolism of ingested proteins.



* includes endogenous amino acids

(Johnson, 1992)

▲ possible microbial action.

Measurement (note faeces and urine both voided via cloaca).

a, amino acid availability
b, ileal amino acid digestibility
c, faecal amino acid digestibility
d, excreta amino acid digestibility.

4.4. Other methods used to asses protein quality

In vitro methods have also been tested to evaluate protein quality. Such methods are generally quicker, cheaper and in the present welfare / animal rights conscious times more acceptable.

Most often enzymes are used in the evaluation of proteins using in vitro methods. In such methods proteolytic enzymes and the feed or raw sample are incubated under a suitable, constant environment. The undigested material is separated from digested material by precipitation, dialysis, and gel or membrane filtration (McNab, 1979). The main enzyme used is pepsin although often a mixture is used (Low, 1990).

McNab (1979) claimed that the methods are inaccurate due to incomplete digestion. However since then there has been more success but there is still much work to be done before the results reflect those obtained in vivo (Low, 1990).

Other methods use chemical analysis especially one devised by Carpenter (1957) in which it was intended to assess lysine availability to the animal. The method used the reactivity of fluoro-2,4-dinitro benzene with the alpha-amino group of lysine. The method has been improved to decrease the time required for the reaction. (Silcock method, Roach *et al.*, 1967). Another method using 2,4,6-trinitrobenzene sulphonic acid was devised by Kakade and Liener (1969). This method has been found to be very accurate and compared well with in vivo results McNab (1979). Little work has been done with other chemical methods for other amino acids.

SECTION B

EXPERIMENTAL WORK

AMINO ACIDS IN WHEAT FED TO BROILER CHICKENS

1. INTRODUCTION

Wheat is a major constituent of poultry diets, typically making up 0.5 of the feed. It therefore can account for 0.8 of ME and 0.4 of protein. Variation in total wheat protein content is due to environmental conditions and the variety, which may lead to differences in productive performances in poultry.

Estimates of the nutritional value of protein are based upon amino acid composition which is traditionally expressed as the total amino acid content. However amino acids are not always available for maintenance and production largely due to incomplete digestion of protein. Amino acids are present in feed as components of protein and are liberated during digestion. However there is large variation between amino acids in terms of the extent to which they are digested. This can result in great differences between amino acids present in the diet and those actually digested.

In this work trials were carried out using wheats of a known variety with either a known protein content or nitrogen fertilizer application. Previous work has failed to investigate the influences of variety and fertilizer levels on the amino acid digestibility. Only recently have these aspects been considered and taken into account. By examining varietal differences and the influence of nitrogen fertilizer it should be possible to identify if there are major differences in amino acid digestibility. Nutritional value of dietary protein is increasingly being expressed in terms of digestible rather than total amino acids. However the traditional use of a constant correction factor to convert total to digestible amino acid may not be valid due to the variation in the individual amino acid digestibility.

2. EXPERIMENTAL WORK

As with any digestibility experiments the basis of the work is to feed the bird a specific diet and collect the excreta. Due to the effect of microbial fermentation and resulting degradation of digesta in the caeca of poultry it was decided to obtain the sample from the ileum. Thus the sample was obtained from the part of the gastrointestinal tract which lay between Meckel's Diverticulum and the ileal- caecal- colonic junction.

The major problems in digestibility experiments are endogenous losses *i.e.* amino acids produced by the bird in the digestive tract which are not of immediate dietary origin. Results which do not account for such losses are termed apparent digestibility. True digestibility values are obtained following correction for these losses. Estimation of these losses is typically through comparing output of a fed bird with that obtained with one that has been starved, with losses from the latter being assumed to be endogenous. However this approach may be criticised on the grounds that net output from a starved bird may not truly representative of endogenous losses from a full-fed bird. One way of eliminating this problem is to feed a nitrogen free diet. Here 1 set of birds is fed a nitrogen free diet and the others are fed diets containing the protein source under investigation. The values obtained for the nitrogen free diets are taken into account when the other diets are analysed. However results show that variation in nitrogen intake itself can alter the endogenous losses.

The technique used in the current project will hopefully solve this problem by taking 3 diets with increasing levels of wheat (the only protein source) in the diets and measuring the amino acid digestion by using regression equations with the values obtained being used to calculate total digestibility.

Young birds, fed *ad libitum,* were used which were obtained from a commercial hatchery at 1 day old. Digesta samples were obtained from the birds at 21 days of age. This age was used as the birds were of sufficient live weight to produce enough sample whilst being in a steady state. A dietary marker, titanium dioxide, was used so that it was not necessary to measure quantitatively the actual feed intake or digesta of the birds.

2.1. TRIALS

The experimental programme extended from autumn 1992 to summer 1995.

2.1.1. Trial 1

In this trial the wheat variety Mercia was investigated. Wheat samples had been grown at the same location and treated with increasing levels of nitrogen fertilizer; 0, 120, and 240 kg/ha.

9 diets (3 per wheat sample) were formulated using 3 inclusion levels of wheat; 250, 500, and 750 g wheat / kg. Other dietary constituents were (per kg) oil (50g) mineral/vitamin (50g) and 5g titanium dioxide. Diets were made up to 1 kg with a 50:50 starch glucose mix.

54 chickens were used in this trial, 6 per diet. These were fed a general chick starter diet until 18 days old when they were fed one of the 9 diets. The birds were held in an environmentally controlled metabolism room and allowed free access to water and feed.

At 21 days the contents of the ileum were removed from the chicken and frozen immediately before being freeze-dried.

2.1.2. Trial 2

Sample sizes obtained in the previous trial were too small for analysis, resulted in them having to be bulked. This was considered unacceptable so a second trial was performed immediately with the primary aim of increasing the sample size obtained from each bird. As with trial 1 the variety Mercia was used which was obtained from the University mill.

3 diets were formulated using one wheat sample at the same three inclusion levels used in trial 1. Other dietary constituents and most of the conditions were as in trial 1. The changes made to the conditions were that the birds were on trial in pairs and 3 h before the sample was collected they were starved of food for 1 hour before being given access to feed once more. Additionally the samples from the pair of birds were collected in the same container. The total transit time for food in the gut of poultry is less than 4 hours, therefore at 2 h after the start of re-feeding there should be an adequate gut fill.

When establishing the pairs, all of the birds were weighed at 14 days of age and placed in a cage with a partner of a similar weight.

2.1.3. Trial 3

Using the conditions determined in trial 2 a further trial was designed to investigate the digestibilities of amino acids in two named varieties. These two varieties, Hussar and Brigadier, each had two protein levels (high and low). 12 diets were formulated and 144 chickens were used. Due to the large number of birds the trial was carried out in two halves, with treatments replicated equally over both.

2.1.4. Trial 4

The first of these trials used two varieties which were isogenic except

for hardness and protein content. The trial was designed in the same way as trial 3 but was conducted over one time period.

Four wheats samples were used in this trial. Two were the parental lines, Hobbit/Avalon 5A where the Avalon 5A substitution has been made and Hobbit 5A. The other two samples were isogenic lines for hard endosperm texture/high protein and soft endosperm texture/low protein (approximately 10 g difference in protein concentration/ kg).

2.1.5. Trial 5

Trial 5 was a further investigation of the genetic aspects of amino acid content and digestibility. Two well established varieties were used, Cappelle and Bezostaya, at three inclusion rates. All conditions were the same as in the previous experiments using 72 birds and 6 diets.

2.1.6. Trial 6

Trial 6 looked at the effects of the introduction of a rye gene (the 1B/1R gene) on both hard and soft wheats. Two hard wheats were investigated (Brigadier 1B/1R and Buster no 1B/1R) and two soft (Riband no 1B/1R and Hunter 1B/1R), so there were two soft wheats (one with the 1B/1R translocation the other without) and two hard wheats (one with the 1B/1R the other without). The procedure followed was identical to trial 4 with 12 diets and 6 pairs of birds per diet.

A summary of trials conducted is presented in Table 1(overleaf).

Table 1 Summary of trials

TRIAL	DETAILS	NO. BIRDS
ONE	MERCIA, 3 Nitrogen levels	54, 6/diet
	0, 120, 240 kg/ha	
TWO	MERCIA	36, 12/diet
THREE	BRIGADIER,	144
	C.P. 85 and 108 g/kg	
	HUSSAR,	
	C.P. 87 and 107 g/kg	
FOUR	HOBBIT (AVALON 5A)	144
	HOBBIT (HOBBIT 5A)	
	HARD ENDOSPERM HIGH PROTEIN	
	SOFT ENDOSPERM LOW PROTEIN	
FIVE	CAPPELLE, C.P. 121.3 g/kg	72
	BEZOSTAYA, C.P. 136.4 g/kg	
SIX	BRIGADIER IB/IR (hard)	144
	BUSTER no IB/IR (hard)	
	RIBAND IB/IR (soft)	
	HUNTER no IB/IR (soft)	

2.2. Chemical Analysis

All of the samples after freeze drying were weighed and ground using a pestle and mortar before being analysed, in duplicate, for amino acid and titanium dioxide content.

Amino acid content was determined after hydrolysing in 6M HCl for 24 hours at 110°C. All samples were oxidised using performic acid to avoid cystine and methionine loss. Samples were assayed on a cation exchange

column with norleucine as internal standard. Titanium was determined using a modification of the AOAC method necessary because of the low sample size. Samples were ashed at 580°C for 13 hours before being dissolved in 7.4M H_2SO_4 and diluted with water and H_2O_2 (30% v/v). Absorbance was measured at 410 nm.

2.3. Determination of coefficient of amino acid digestibility.

The titanium and amino acid results from the trials were used to calculate the apparent amino acid digestibility using the following equation:

$$1-(aa_{dig} * marker_{feed}) / (aa_{feed} * marker_{diet})$$

(where aa = amino acid and dig = digesta)

2.4. Apparent digestible amino acid content of diet and wheat

This was determined by multiplying the apparent digestibility coefficient, for that inclusion rate, by the amount of the amino acid in that particular diet. The amount of apparent digestible amino acid was plotted against the inclusion of wheat in the diet and using regression a line of best fit was inserted (see figure 2). This was carried out for all of the amino acids and the apparent amino acid digestibility coefficient was obtained using the regression equations. The true digestibility coefficient was determined by adding the intercept to the amount of apparent digestible amino acid.

Example of calculation: Wheat B1 Threonine

Content of apparent digestible amino acid (DAA) in diets with wheat (g/kg diet)

Mean values:	wheat inclusion (g/kg)	DAA. (g/kg)
	250	0.1056
	500	0.3902
	750	0.7248

The mean DAA. values were plotted against the inclusion rate of wheat (fig 2).

Regression equation:

y = a *x + b (a = gradient, b = y intercept)

y = (0.001238 *x) +(-0.21233) (R = 0.997)

Therefore:

if x =1000 (wheat)

y (DAA.) = 1.02567 (g digestible threonine / kg wheat)

Coefficient of DAA:

1.02567 / 2.19 (2.19 = g/kg threonine in wheat) = 0.468

For true digestibility the Y intercept (0.21233) is deducted:

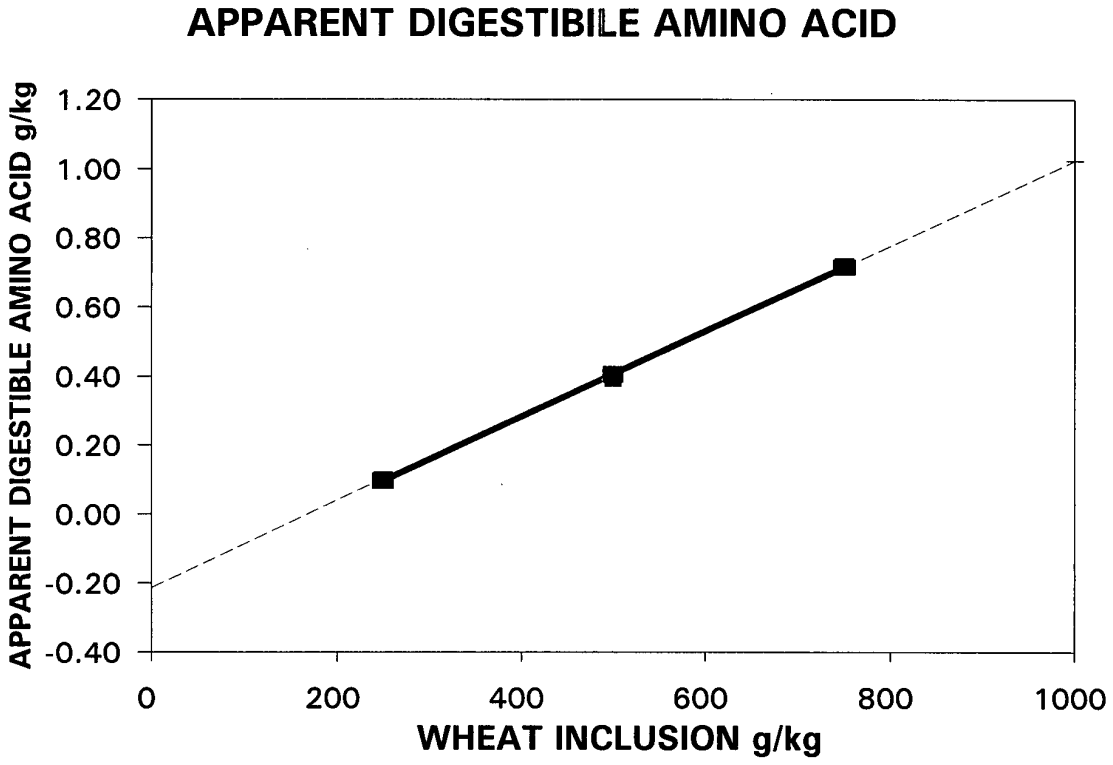
ie 1.02567 - (- 0.21233) = 1.238 g/kg

(Note that the negative term for the intercept in fact means that it is added)

Coefficient of true digestibility:

1.238 / 2.19 = 0.566

Figure 2 Wheat inclusion and apparent digestible amino acid content for a specific amino acid (threonine).



3. RESULTS AND DISCUSSION

The results for all of the trials are presented in the appendix. Abbreviations used are listed below.

Table 2 Abbreviations used in results

ABBREVIATION	MEANING
AA	amino acid
A	0 kg/Ha N fertilizer
B	120 kg/Ha N fertilizer
C	240 kg/Ha N fertilizer
B1	Brigadier 1
BH	Brigadier high
H1477	Hussar 1477
H1398	Hussar 1398
Wheat 1	Hard/low protein
Wheat 2	Soft/high protein
Wheat 3	Hobbit/Avalon 5a
Wheat 4	Hobbit/Hobbit 5a
Cap	Cappelle
Best	Bezostaya
5002	Hard 1B1R
5005	Soft no 1B1R
5011	Hard no 1B1R
5020	Soft 1B1R

Table 3 Abbreviations for amino acids

ABBREVIATION	AMINO ACID
CYS	CYSTINE
MET	METHIONINE
ASP	ASPARTIC ACID
THR	THREONINE
SER	SERINE
GLU	GLUTAMIC ACID
GLY	GLYCINE
ALA	ALANINE
VAL	VALINE
ILE	ISOLEUCINE
LEU	LEUCINE
TYR	TYROSINE
PHE	PHENYLALANINE
HIS	HISTIDINE
LYS	LYSINE
ARG	ARGININE
PRO	PROLINE

3.1. Trials

3.1.1. Trial 1

Results from trial 1 for the mean apparent digestible amino acid content are shown in table 5 for the 3 inclusion levels. The regression data obtained from these results appear in tables 11-13 which also shows the total amount of amino acid in the wheat at each fertilizer level.

The apparent digestibility coefficients vary suggesting that the practice of using a constant conversion factor to calculate the amount of digestible amino acid in a wheat sample is invalid. Results suggest that, as the N level increases, the apparent digestibility increases for the essential amino acids, whereas the values for the nonessential amino acids remain relatively constant. However data for wheat B (120 Kg/Ha) appear to be lower than for the other 2 wheats, which can be seen in figure 3.

Statistical analysis revealed that there were significant differences between the wheats, individual amino acids and the rate of inclusion of the wheat (see table 32).

3.1.2. Trial 2

Table 6 shows the content of digestible amino acid in the three diets and the regression data (table 14) confirms that the response to increasing levels of wheat is linear. Again there were significant differences between the inclusion levels and the individual amino acids (table 33) and it was confirmed that the use of a constant conversion factor to estimate the level of digestible individual amino acid in a wheat sample for poultry feed is invalid.

3.1.3. Trial 3

This trial investigated the influence of wheat variety and crude protein on amino acid digestibility The content of apparent digestible amino acids in the diets is shown in table 7 and regression analyses in tables 15-18. Significant differences between the 2 varieties were revealed and the level of crude protein also produced significant differences (table 34).

Individual amino acids had varying digestibility values but, as the crude protein increased, the digestibility generally increased (table 7 and figure 4). It was also observed that the difference between true and apparent digestibility varied for individual amino acids (see figure 5) .

The first 3 trials used named varieties of wheats and differences in results obtained could only be described as a being due to the effect of crude protein or N fertilizer levels. Protein levels and therefore amino acid concentrations in wheat are influenced by many factors not just environmental ones. The programme had intended to examine possible varietal differences in terms of apparent amino acid digestibility. However attempting to identify possible influences of genotype simply by referring to "named" varieties was at this stage considered inappropriate. This was because reliance on "name" alone gives no guidance as to the genetic relationship. Two "named" varieties for example could be distantly or closely related, but the name alone would give no guidance on this. Accordingly it was therefore thought that a more accurate investigation into the digestibility of amino acids in wheat would be obtained by looking at wheat samples with a similar genetic background with one or two genetic differences (ie. near isogenic lines) instead of named varieties. Thus differences in results could be attributed to the presence of specific characteristics.

3.1.4. Trial 4

The apparent digestibility of the amino acids in the wheats analysed are shown in table 8. As expected there is little variation in the nitrogen and therefore, crude protein levels (table 30). There were significant differences between the wheat samples, inclusion levels and individual amino acids (table 35).

Regression data (tables 19-22) revealed that the apparent digestibility of the amino acids still increased linearly with the inclusion levels.

There did appear to be a greater difference in the amino acid digestibility coefficients between the wheats compared to earlier trials, see tables 19-22. The higher protein hard wheat, wheat 1, had a higher digestibility compared to the lower protein soft wheat (wheat 2). The parent variety with the Avalon 5A substitition, wheat 3, had some of the lowest digestibility suggesting that the Avalon 5A may decrease the digestibility. This is evident when looking at figure 6.

3.1.5. Trial 5

The 2 well-established varieties, Cappelle and Bezostaya, were also significantly different from each other as shown in table 36 and significant differences between inclusion rates and individual amino acids were also observed. The apparent digestible amino acid content for the two varieties at each inclusion level is shown in table 9 and regression analyses in tabes 23 and 24. Cappelle, despite a lower crude protein concentration, appeared to have a particularly high glutamate level compared to Bezostaya and, although it was used as a feed wheat, had lower digestibility values, as displayed in table and shown in figure 7.

3.1.6. Trial 6

This trial also revealed that there were significant differences between the wheat samples themselves as well as individual amino acids and inclusion levels (table 37). Apparent digestible amino acid values in each diet are shown in table 10 , and the regression data (tables 25-28) reveal that the amount of digestible amino acid increases in a linear fashion as the inclusion level increases. The apparent digestibility values for several amino acids (shown in figure 8) revealed that although there were no apparent significant differences as a result of the hard / soft characteristic, the presence of the rye gene (1B/1R) did appear to decrease the digestibility.

Figure 3

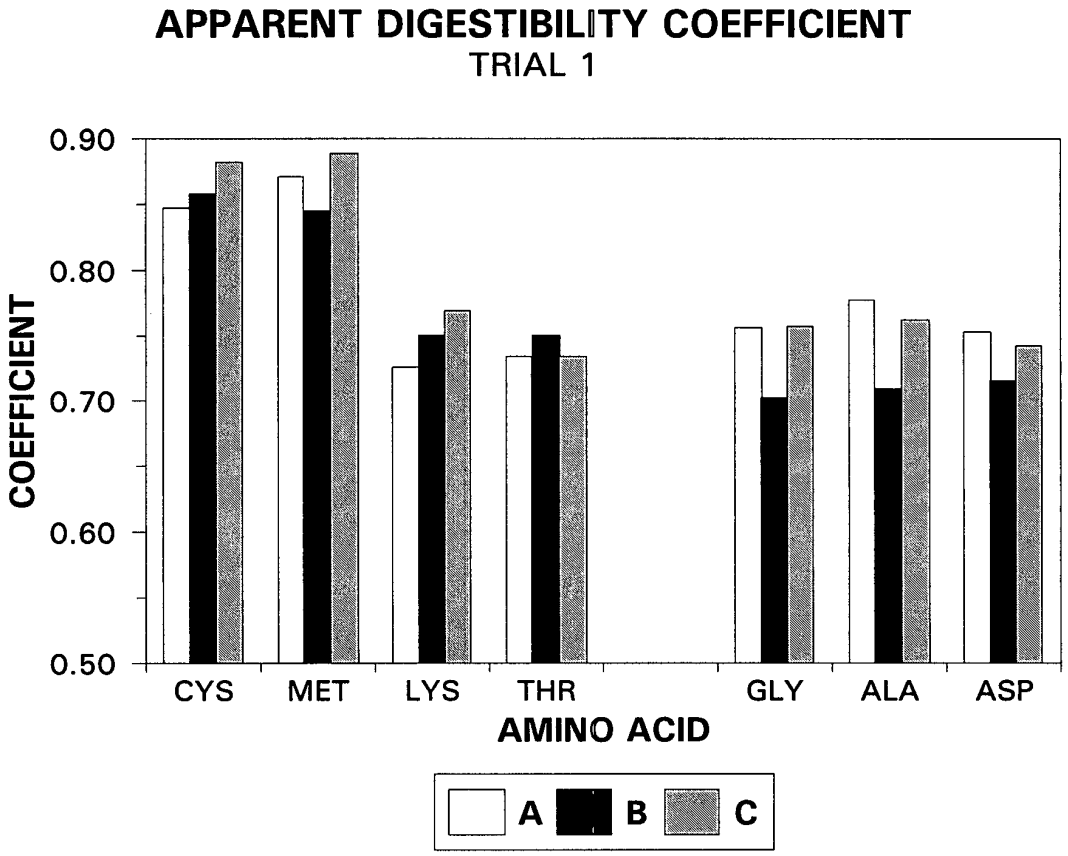

APPARENT DIGESTIBILITY COEFFICIENT
TRIAL 1
COEFFICIENT
0.90
0.80
0.70
0.60
0.50
CYS
MET
LYS
THR
GLY
ALA
ASP
AMINO ACID
A
B
C

Figure 4

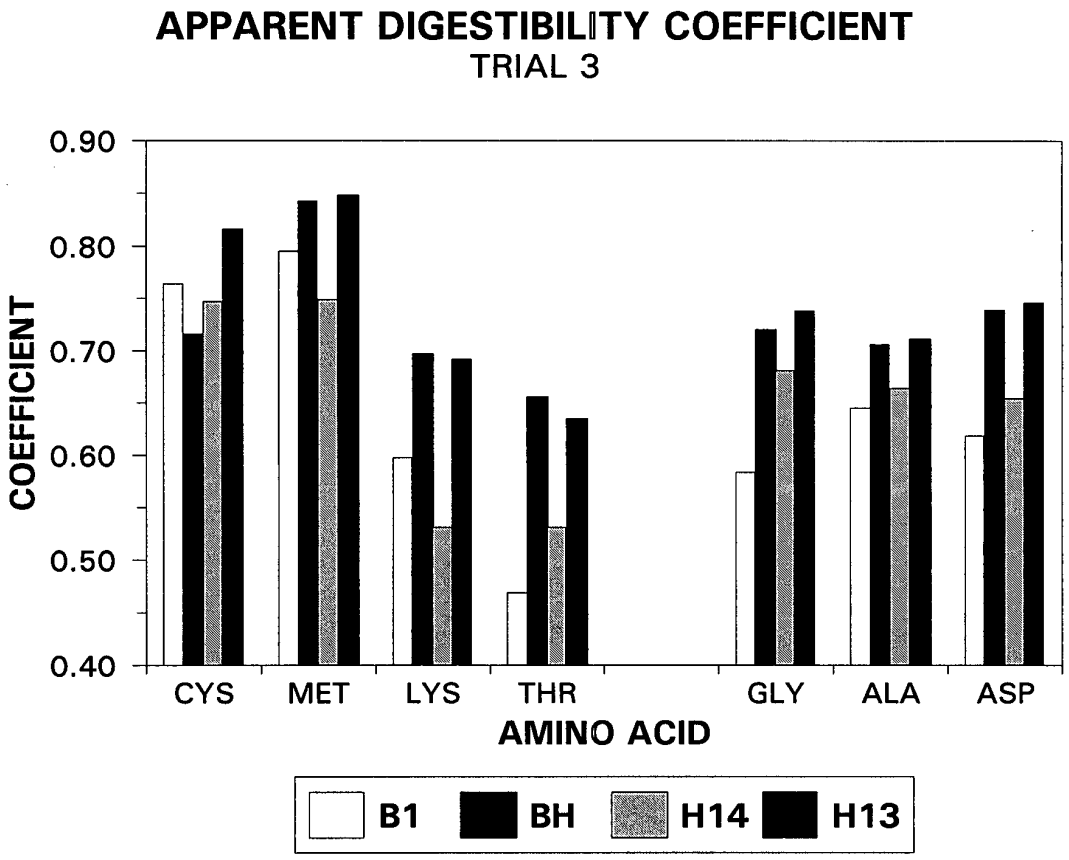

APPARENT DIGESTIBILITY COEFFICIENT
TRIAL 3
COEFFICIENT
0.90
0.80
0.70
0.60
0.50
0.40
CYS
MET
LYS
THR
GLY
ALA
ASP
AMINO ACID
B1
BH
H14
H13

Figure 5

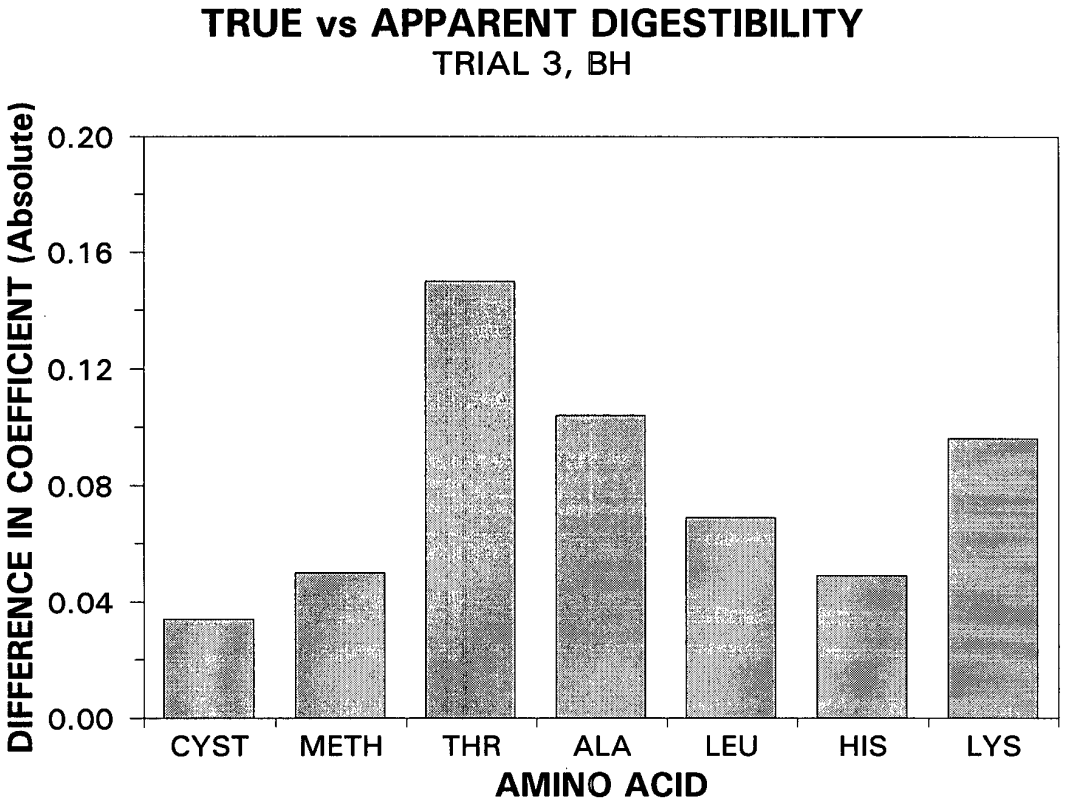

TRUE vs APPARENT DIGESTIBILITY
TRIAL 3, BH
DIFFERENCE IN COEFFICIENT (Absolute)
0.20
0.16
0.12
0.08
0.04
0.00
CYST
METH
THR
ALA
LEU
HIS
LYS
AMINO ACID

Figure 6

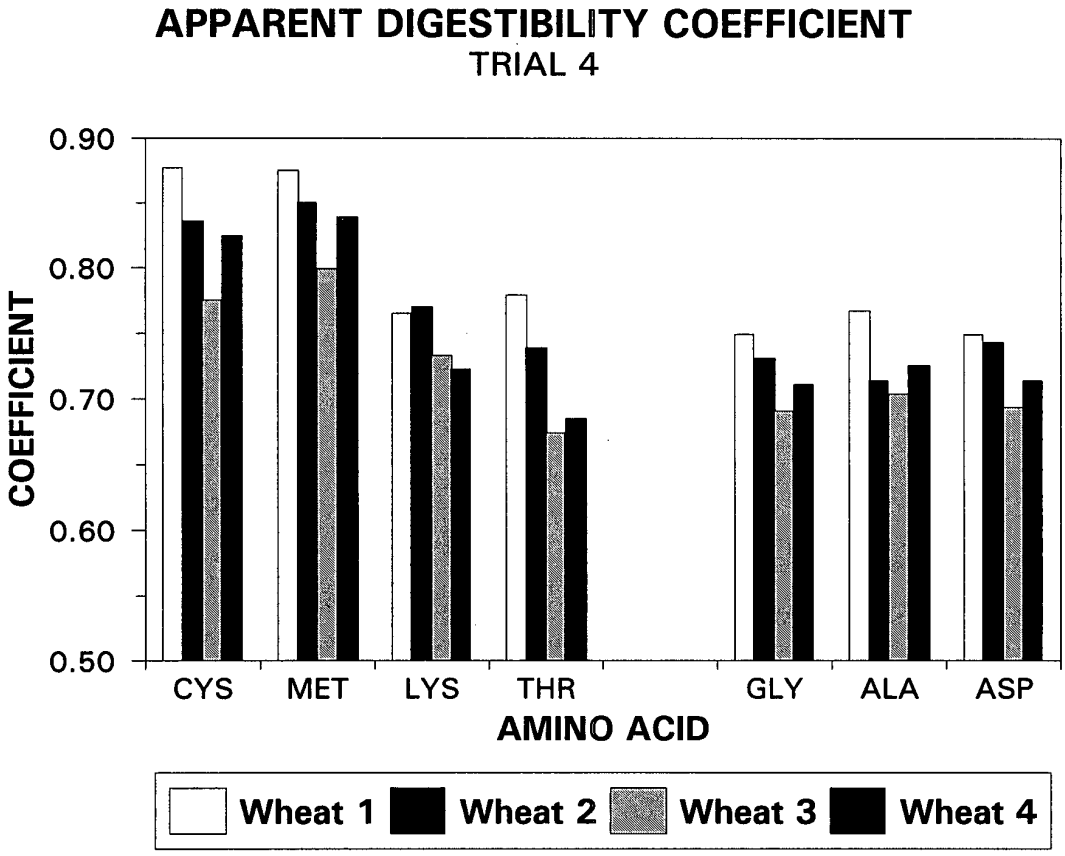

APPARENT DIGESTIBILITY COEFFICIENT
TRIAL 4
0.90
0.80
0.70
0.60
0.50
COEFFICIENT
CYS
MET
LYS
THR
GLY
ALA
ASP
AMINO ACID
Wheat 1
Wheat 2
Wheat 3
Wheat 4

Figure 7

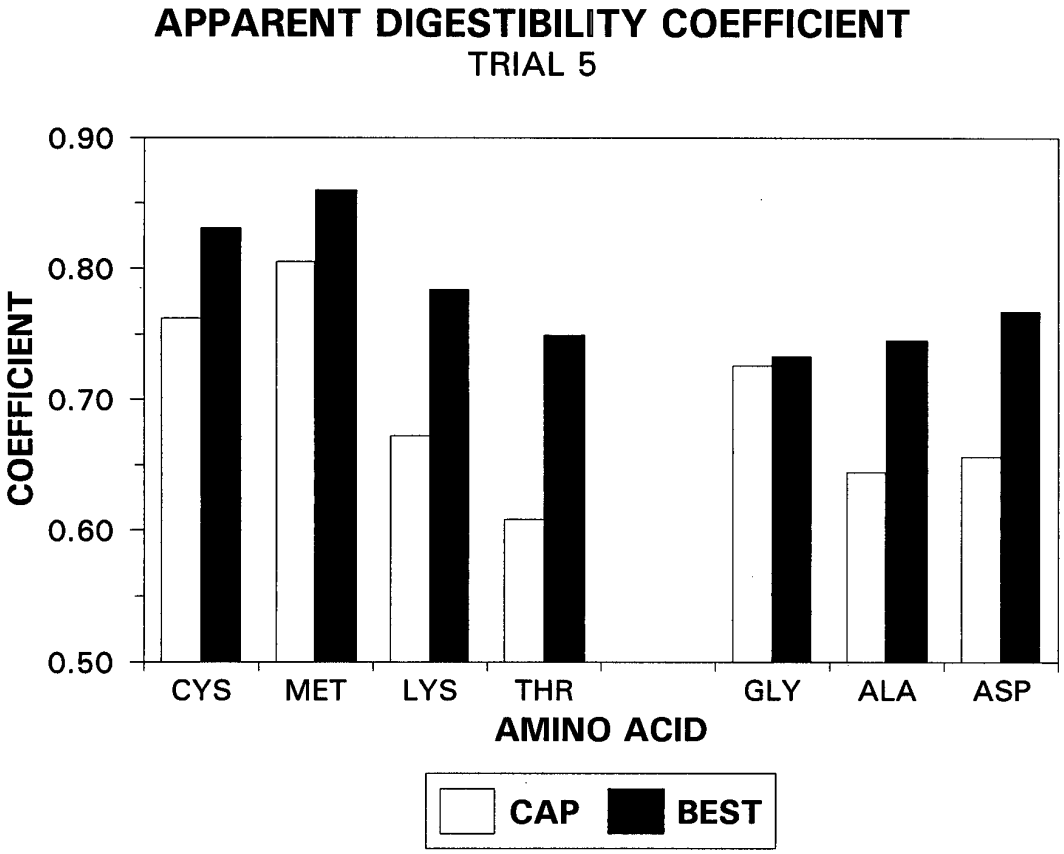
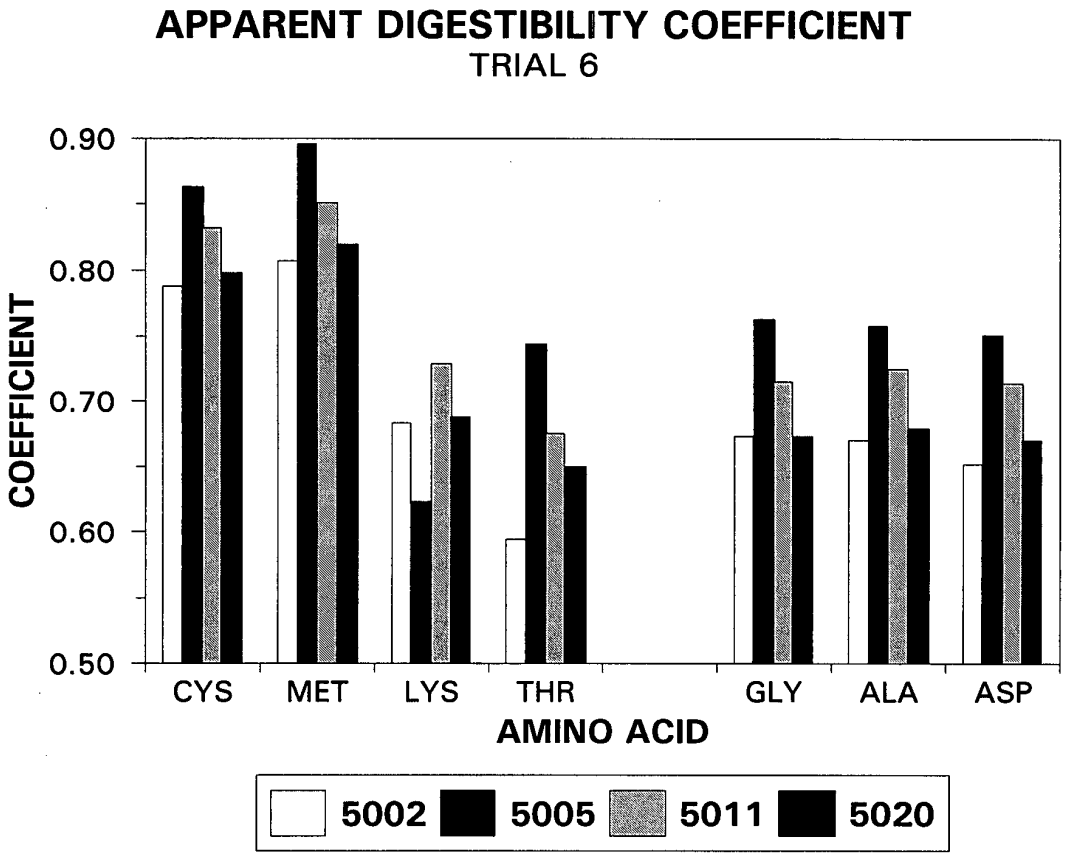

APPARENT DIGESTIBILITY COEFFICIENT
TRIAL 5
0.90
0.80
0.70
0.60
0.50
COEFFICIENT
CYS
MET
LYS
THR
GLY
ALA
ASP
AMINO ACID
CAP
BEST

Figure 8


APPARENT DIGESTIBILITY COEFFICIENT
TRIAL 6
COEFFICIENT
0.90
0.80
0.70
0.60
0.50
CYS
MET
LYS
THR
GLY
ALA
ASP
AMINO ACID
5002
5005
5011
5020

3.2. Nitrogen content and content of digestible amino acid.

The data generated presented a useful opportunity to estimate whether there was any correlation between grain nitrogen content and the content of digestible amino acids. This was examined for lysine and threonine (2 nutritionally essential amino acids) and glutamic acid (non-essential). Results are presented in figures 9, 10 and 11 respectively. The responses obtained indicate that there is no significant correlation, although for lysine and threonine they may be some evidence of an increase in digestible threonine and lysine as the nitrogen content increases.

Table 4 Key for graphs presenting digestible amino acid content

SYMBOL	REPRESENTS
1	TRIAL 1 FERT A
2	TRIAL 1 FERT B
3	TRIAL 1 FERT C
4	TRIAL 3 BRIGADIER 1
5	TRIAL 3 BRIGADIER HIGH
6	TRIAL 3 HUSSAR 1398
7	TRIAL 3 HUSSAR 1477
8	TRIAL 4 WHEAT 1
9	TRIAL 4 WHEAT 2
10	TRIAL 4 WHEAT 3
11	TRIAL 4 WHEAT 4
12	TRIAL 5 CAPPELLE
13	TRIAL 5 BEZOSTAYA
14	TRIAL 6 5002
15	TRIAL 6 5005
16	TRIAL 6 5011
17	TRIAL 6 5020

Figure 9

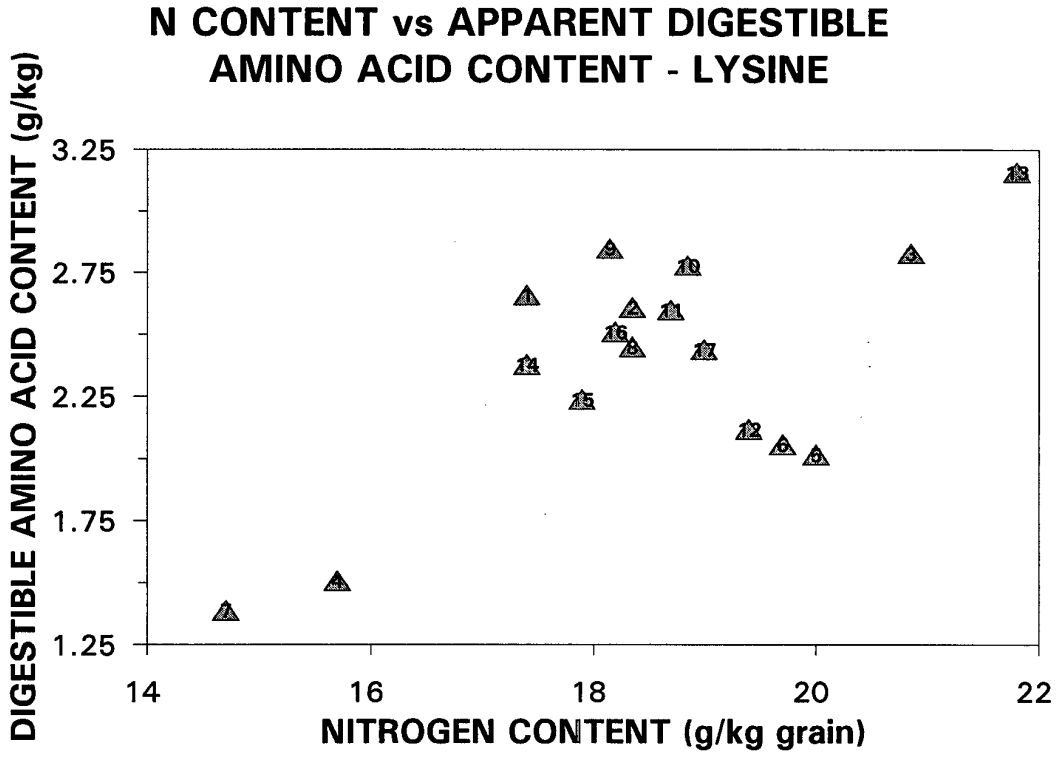

N CONTENT vs APPARENT DIGESTIBLE AMINO ACID CONTENT - LYSINE
DIGESTIBLE AMINO ACID CONTENT (g/kg)
3.25
2.75
2.25
1.75
1.25
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 10

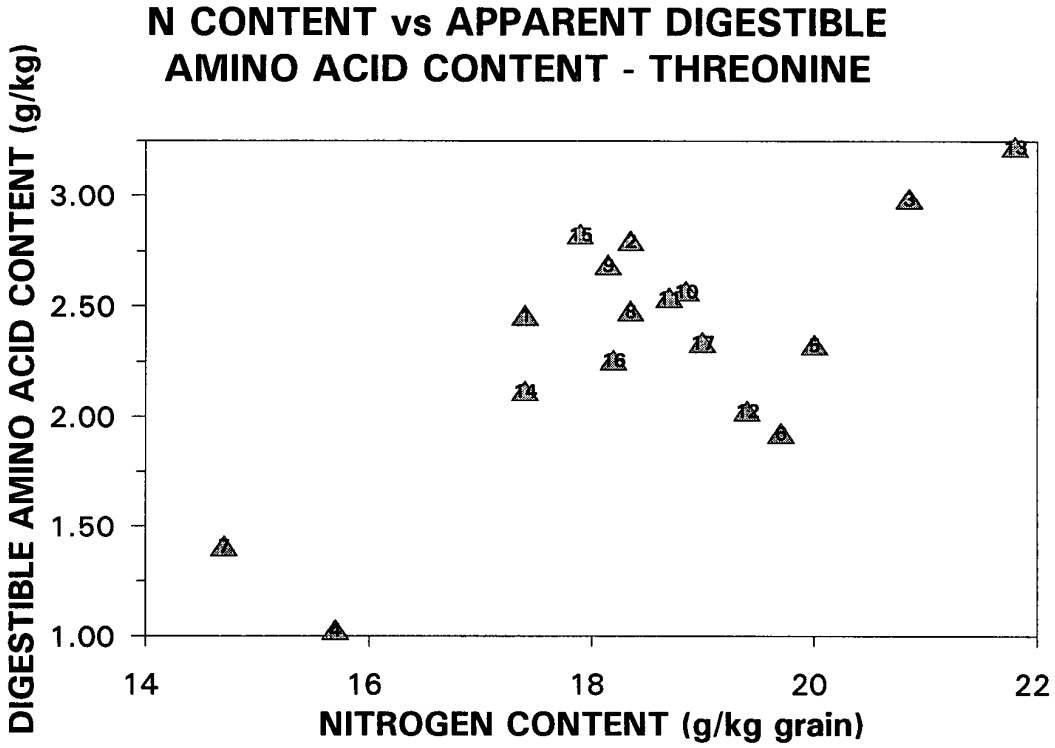
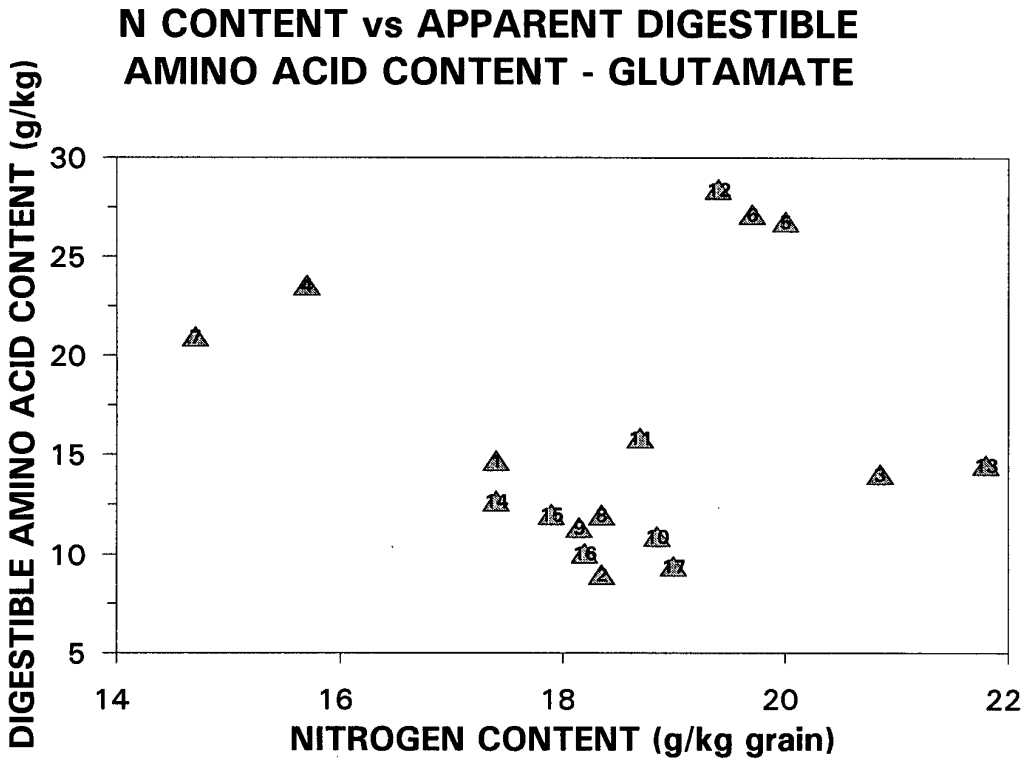

N CONTENT vs APPARENT DIGESTIBLE AMINO ACID CONTENT - THREONINE
DIGESTIBLE AMINO ACID CONTENT (g/kg)
3.00
2.50
2.00
1.50
1.00
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 11


N CONTENT vs APPARENT DIGESTIBLE AMINO ACID CONTENT - GLUTAMATE
DIGESTIBLE AMINO ACID CONTENT (g/kg)
30
25
20
15
10
5
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

3.3 Coefficient of apparent digestibility and N content

The relationship was examined further by relating the coefficient of digestibility of amino acids with grain nitrogen content. Responses obtained are presented in figures 12, 13, 14, 15 and 16 respectively for cystine, lysine, methionine, threonine and glutamic acid. This analysis is perhaps more meaningful as it removes variation attributable to differences in total amino acid content.

Results indicated that no correlation was apparent for cystine or glutamic acid, but that a significant ($P < 0.05$) positive response was obtained for lysine, threonine and methionine.

Regression equations

Lysine	$y = x * 0.0184 + 0.3694$
Methionine	$y = x * 0.0121 + 0.6136$
Threonine	$y = x * 0.0243 + 0.2208$

Studies currently in progress will contribute to this analysis by providing more data points.

Figure 12

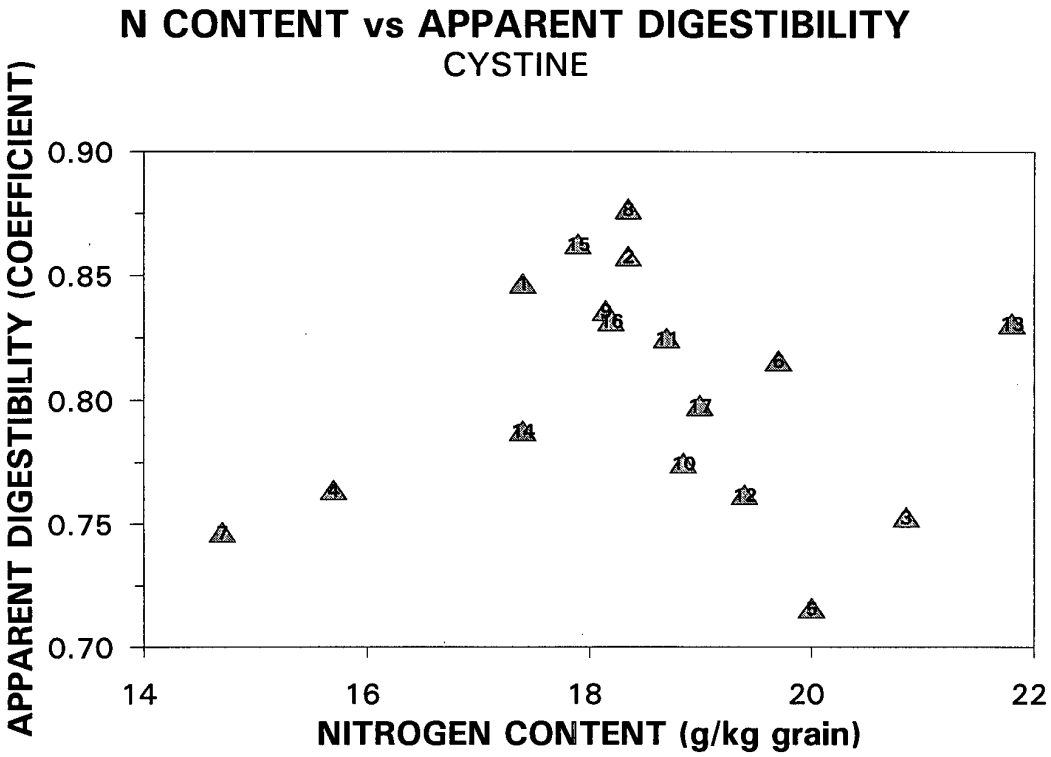

N CONTENT vs APPARENT DIGESTIBILITY
CYSTINE
APPARENT DIGESTIBILITY (COEFFICIENT)
0.90
0.85
0.80
0.75
0.70
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 13

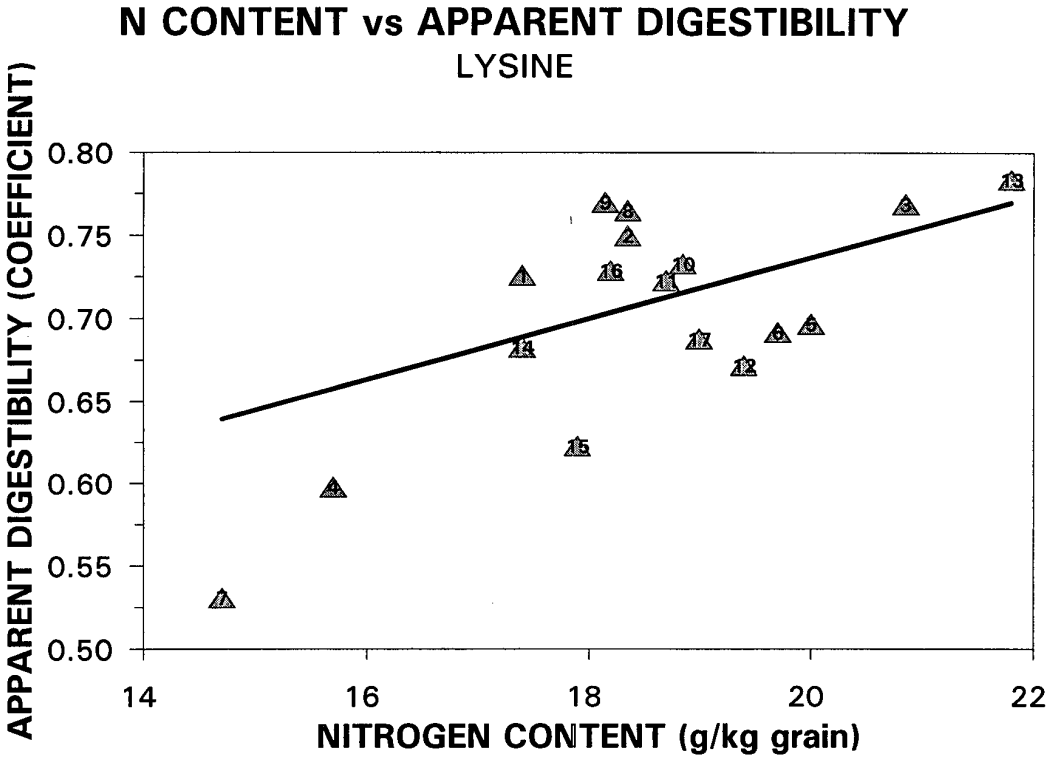

N CONTENT vs APPARENT DIGESTIBILITY
LYSINE
APPARENT DIGESTIBILITY (COEFFICIENT)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 14

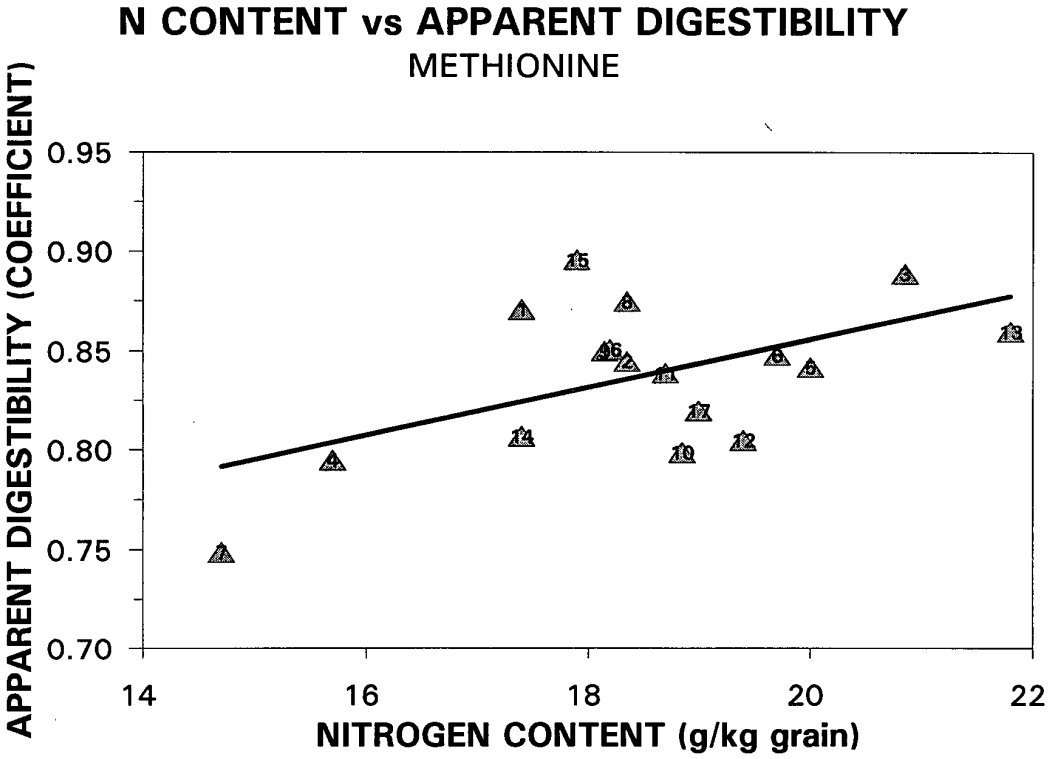

N CONTENT vs APPARENT DIGESTIBILITY
METHIONINE
APPARENT DIGESTIBILITY (COEFFICIENT)
0.95
0.90
0.85
0.80
0.75
0.70
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 15

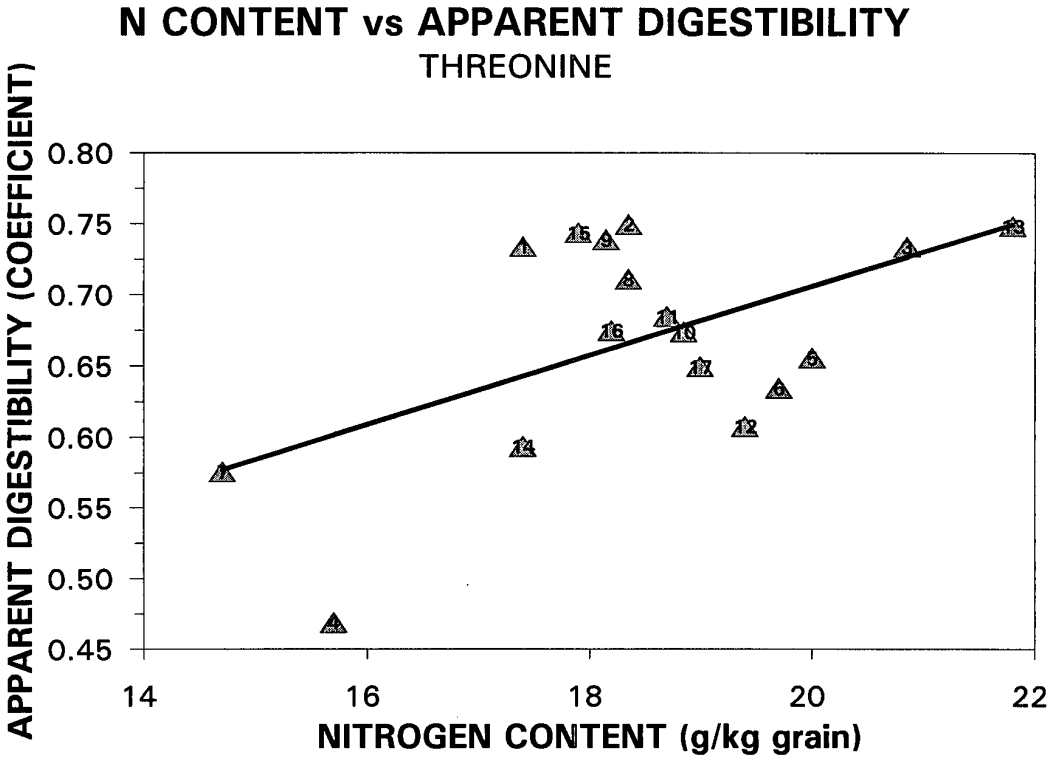
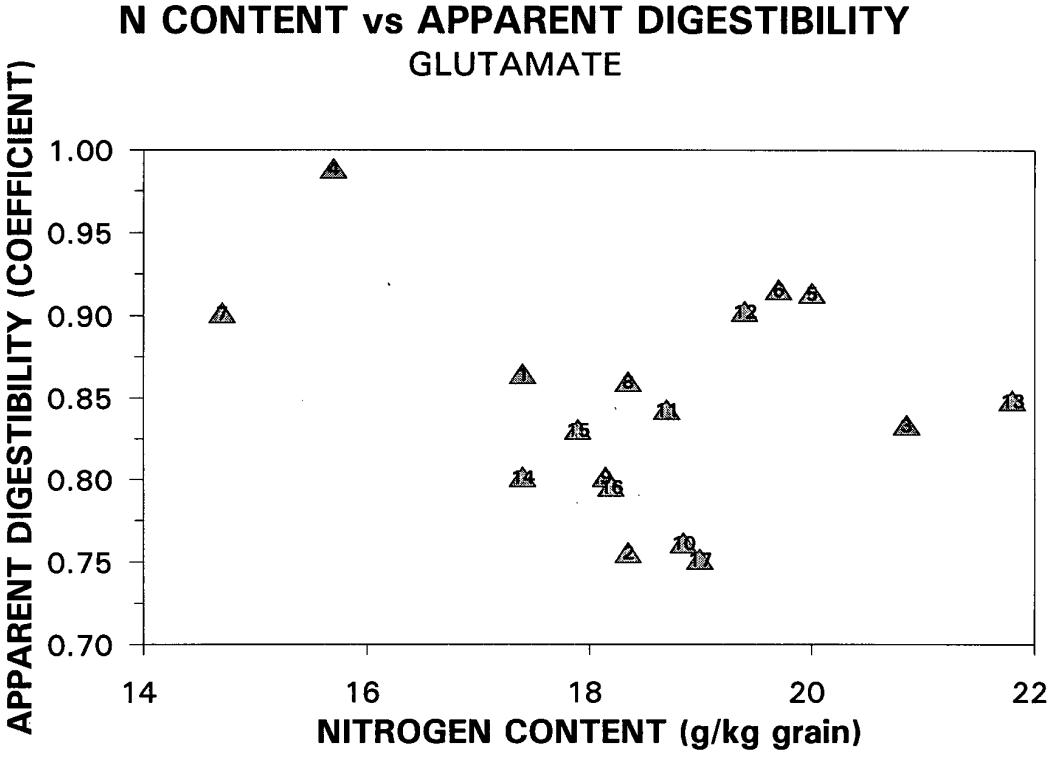

N CONTENT vs APPARENT DIGESTIBILITY
THREONINE
APPARENT DIGESTIBILITY (COEFFICIENT)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

Figure 16


N CONTENT vs APPARENT DIGESTIBILITY
GLUTAMATE
APPARENT DIGESTIBILITY (COEFFICIENT)
1.00
0.95
0.90
0.85
0.80
0.75
0.70
14
16
18
20
22
NITROGEN CONTENT (g/kg grain)

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5. APPENDICES

Table 5 Apparently Digestible Amino Acids (G/kg)from Wheat Grown At 3 Levels of Nitrogen Fertilizer (A: 0, B 120, C 240kg/ha)

TRIAL 1	RATE OF INCLUSION OF WHEAT (g/kg)												
AMINO ACIDS	250			500			750			MEAN FERTILIZER			MEAN
	A	B	C	A	B	C	A	B	C	A	B	C	
CYS	0.44	0.39	0.70	1.01	1.21	1.56	1.60	1.81	2.31	1.01	1.14	1.52	1.22
MET	0.32	0.33	0.44	0.71	0.83	0.97	1.11	1.21	1.45	0.71	0.79	0.95	0.82
ASP	0.71	0.39	0.97	1.90	2.18	2.65	3.22	3.12	3.80	1.94	1.90	2.47	2.10
THR	0.30	-0.01	0.43	0.94	1.23	1.41	1.76	1.79	2.11	1.00	1.00	1.31	1.10
SER	0.75	0.52	1.00	1.86	2.15	2.55	3.07	3.17	3.75	1.90	1.95	2.44	2.09
GLU	3.20	1.34	3.10	6.99	4.47	7.07	10.88	6.31	10.31	7.03	4.04	6.82	5.96
GLY	0.55	0.31	0.78	1.38	1.44	1.98	2.30	2.05	2.82	1.41	1.27	1.86	1.51
ALA	0.59	0.37	0.77	1.42	1.47	1.91	2.36	2.08	2.73	1.45	1.31	1.80	1.52
VAL	0.57	0.37	0.87	1.45	1.56	2.20	2.45	2.24	3.17	1.49	1.39	2.08	1.66
ILE	0.62	0.50	0.89	1.46	1.63	2.11	2.36	2.38	3.12	1.48	1.51	2.04	1.68
LEU	1.23	1.05	1.72	2.81	3.07	3.98	4.48	4.48	5.84	2.84	2.87	3.85	3.19
TYR	0.46	0.44	0.61	1.06	1.26	1.47	1.66	1.80	2.11	1.06	1.17	1.40	1.21
PHE	0.99	0.94	1.45	2.53	2.50	3.21	3.47	3.68	4.74	2.33	2.37	3.12	2.61
HIS	0.51	0.44	0.70	1.15	1.30	1.62	1.84	1.87	2.36	1.17	1.20	1.56	1.31
LYS	0.53	0.36	0.56	1.23	1.26	1.45	1.99	1.82	2.04	1.25	1.45	1.35	1.25
ARG	0.96	0.81	1.22	2.21	2.43	2.92	3.53	3.42	4.16	2.23	2.22	2.77	2.41
PRO	1.03	0.71	0.56	2.47	3.15	2.05	4.73	4.79	2.83	2.74	2.89	1.81	2.48
MEAN	0.81	0.55	0.99	1.92	1.95	2.42	0.99	2.41	3.51	1.94	1.77	2.3	
MEAN			0.78			2.10			3.15				2.01

Table 6 Apparently Digestible Amino Acids (g/kg)

TRIAL 2	INCLUSION OF WHEAT g/kg			
AMINO ACIDS	250	500	750	MEAN
CYS	0.98	1.92	2.70	1.87
MET	0.58	1.27	2.00	1.28
ASP	1.87	4.13	5.59	4.19
THR	0.93	2.10	3.46	2.16
SER	1.70	3.70	5.86	3.75
GLU	10.50	12.50	14.78	12.60
GLY	1.59	3.05	4.28	2.98
ALA	1.29	2.80	4.40	2.83
VAL	1.89	3.51	5.11	3.50
ILE	1.42	3.07	4.81	3.10
LEU	2.90	5.53	8.27	5.57
PHE	1.95	4.12	6.40	4.16
HIS	2.08	2.82	3.57	2.83
LYS	0.71	1.84	3.34	1.97
ARG	1.84	4.26	6.74	4.28
PRO	6.34	7.86	9.42	7.87
MEAN	5.73	4.03	2.41	4.06

Table 7 Apparently Digestible Amino Acids From 4 Wheat Samples B1, Bh Brigadier CP 102, 130g/kg, H1398, H1477, Hussar CP 128, 96g/kg

TRIAL 3	RATE OF INCLUSION																
AMINO ACIDS	250				500				750				MEAN				
	B1	BH	H1398	H1477	B1	BH	H1398	H1477	B1	BH	H1398	H1477	B1	BH	H1398	H1477	
CYS	0.34	0.36	0.46	0.31	0.79	0.85	1.05	0.68	1.22	1.26	1.63	1.13	0.79	0.82	1.04	0.71	0.84
MET	0.26	0.29	0.32	0.27	0.54	0.63	0.67	0.49	0.79	1.01	1.04	0.75	0.53	0.64	0.67	0.50	0.59
ASP	0.48	0.46	1.02	0.56	1.28	1.25	2.32	1.29	2.06	2.67	3.77	2.34	1.27	1.46	2.37	1.40	1.63
THR	0.11	0.15	0.27	0.15	0.39	0.75	0.81	0.50	0.72	1.34	1.38	1.01	0.41	0.75	0.82	0.55	0.63
SER	0.33	0.59	0.76	0.49	0.91	1.58	1.68	1.12	1.49	2.59	2.72	1.68	0.91	1.58	1.72	1.10	1.33
GLU	4.86	6.09	6.48	4.96	10.14	12.79	13.23	10.04	15.40	19.95	20.32	15.70	10.1	12.94	13.3	10.23	11.66
GLY	0.38	0.44	0.58	0.46	1.02	1.21	1.51	1.00	1.43	2.12	2.24	1.80	0.94	1.26	1.44	1.08	1.18
ALA	0.43	0.37	0.51	0.43	0.97	1.07	1.18	0.90	1.53	1.85	1.96	1.60	0.97	1.10	1.22	0.98	1.07
VAL	0.47	0.50	0.62	0.47	1.15	1.33	1.44	1.03	1.61	2.35	2.39	1.61	1.08	1.39	1.48	1.04	1.25
ILE	0.58	0.75	0.69	0.66	1.29	1.78	1.53	1.17	1.97	2.84	2.38	2.20	1.28	1.79	1.53	1.34	1.49
LEU	0.94	1.09	1.29	1.01	2.10	2.61	2.74	2.07	3.21	4.20	4.37	3.32	2.09	2.63	2.80	2.13	2.41
TYR	0.09	0.02	0.16	0.08	0.30	0.15	0.36	0.18	0.41	0.37	0.64	0.42	0.26	0.18	0.39	0.23	0.27
PHE	0.47	0.64	0.81	0.55	1.09	1.54	1.71	1.14	1.65	2.51	2.73	1.93	1.07	1.56	1.75	1.21	1.40
HIS	0.29	0.29	0.37	0.28	0.67	0.77	0.84	0.58	1.02	1.14	1.04	1.00	0.66	0.73	0.75	0.62	0.69
LYS	0.32	0.29	0.40	0.36	0.75	0.88	0.93	0.72	1.10	1.44	1.52	1.04	0.73	0.87	0.95	0.71	0.81
ARG	0.66	0.62	0.88	0.69	1.44	1.65	1.85	1.36	2.20	2.73	3.08	2.21	1.43	1.67	1.94	1.42	1.61
PRO	1.76	2.22	2.45	1.39	3.96	4.40	5.05	3.10	6.50	7.70	8.33	5.08	4.07	4.77	5.28	3.19	4.33
MEAN	0.75	0.89	1.06	0.77	1.69	2.07	2.29	1.61	2.61	3.42	3.62	2.64	1.68	2.13	2.32	1.67	
MEAN	0.87				1.92				3.07								1.95

Table 8 Apparently Digestible Amino AcidsFrom: 1 Hard Low Protein, 2 Soft High Protein Nearisogenics, 3 Hobbitt/avalon 5a, 4 Hobbit/hobbit 5a Parental Lines

TRIAL 4	RATE OF WHEAT INCLUSION																
	250				500				750				MEAN				
AMINO ACIDS	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	MEAN
CYS	0.64	0.54	0.83	0.62	1.74	1.27	1.35	1.28	2.24	1.92	1.93	2.02	1.54	1.24	1.37	1.31	1.36
MET	0.37	0.37	0.57	0.42	0.97	0.80	0.88	0.82	1.21	1.20	1.26	1.26	0.85	0.79	0.90	0.83	0.84
ASP	0.84	0.99	1.61	1.10	2.77	2.37	2.72	2.23	3.32	3.61	3.73	3.55	2.31	2.32	2.69	2.30	2.40
THR	0.33	0.39	0.76	0.52	1.42	1.19	1.45	1.15	1.67	1.91	1.95	1.88	1.14	1.17	1.39	1.18	1.22
SER	0.77	0.92	1.49	1.01	2.28	2.11	2.40	2.07	2.84	3.23	3.45	3.22	1.96	2.09	2.45	2.10	2.15
GLU	2.42	2.60	3.80	3.94	6.67	5.67	6.04	7.74	8.53	8.42	8.60	11.92	5.88	5.56	6.15	7.87	6.36
GLY	0.61	0.69	1.11	0.99	1.95	1.63	1.86	1.67	2.33	2.45	2.55	2.68	1.63	1.59	1.84	1.78	1.71
ALA	0.63	0.94	1.14	0.83	1.97	1.65	1.88	1.59	2.36	2.49	2.58	2.51	1.65	1.69	1.87	1.64	1.71
VAL	0.71	0.73	1.17	0.98	2.05	1.74	1.99	1.93	2.63	2.65	2.74	3.10	1.83	1.70	1.97	2.00	1.88
ILE	0.70	0.75	1.14	0.83	1.98	1.67	1.85	1.67	2.43	2.53	2.62	2.58	1.70	1.65	1.87	1.69	1.73
LEU	12.34	1.44	2.13	1.66	3.67	3.13	3.39	3.29	4.57	4.72	4.85	5.07	3.20	3.10	3.46	3.34	3.27
TYR	0.34	0.24	0.57	0.43	1.00	0.55	0.93	0.83	1.21	0.83	1.28	1.30	0.85	0.54	0.93	0.85	0.79
PHE	0.90	0.78	1.39	1.09	2.43	1.73	2.21	2.16	3.04	2.62	3.16	3.32	2.12	1.71	2.26	2.19	2.07
HIS	0.50	0.51	1.09	0.65	1.56	1.16	1.37	1.26	2.18	1.74	1.92	1.99	1.41	1.13	1.46	1.30	1.33
LYS	0.49	0.66	0.99	0.69	1.45	1.43	1.57	1.05	1.73	2.11	2.19	2.03	1.22	1.40	1.58	1.26	1.36
ARG	1.01	1.10	1.80	1.27	2.89	2.41	2.86	2.39	3.34	3.55	3.96	3.72	2.41	2.35	2.87	2.46	2.52
PRO	1.77	1.84	2.72	2.07	4.45	3.90	4.28	4.11	5.74	5.93	6.20	6.32	3.99	3.89	4.40	4.17	4.11
MEAN	0.84	0.91	1.43	1.12	2.43	2.03	2.30	2.20	3.03	3.05	3.23	3.44	2.10	2.00	2.32	2.25	
MEAN				1.08				2.24				3.19					2.17

Table 9 Apparently Digestible Amino Acids From Capelle (Cap) and Bezostaya (Bes) Wheat Varieties

TRIAL 5	RATE OF INCLUSION (g/kg)								
AMINO ACIDS	250		500		750		MEAN		MEAN
	CAP	BES	CAP	BES	CAP	BES	CAP	BES	
CYS	0.48	0.63	0.94	1.43	1.57	2.11	1.00	1.39	1.20
MET	0.37	0.50	0.72	1.06	1.12	1.55	0.73	1.04	0.89
ASP	0.83	1.23	1.63	2.96	2.71	4.29	1.72	2.82	2.27
THR	0.36	0.56	0.89	1.57	1.48	2.31	0.91	1.48	1.20
SER	0.79	1.16	1.69	2.70	2.62	4.06	1.70	2.64	2.17
GLU	7.02	3.30	13.88	7.26	21.36	10.71	14.09	7.09	10.59
GLY	0.37	0.75	1.32	1.80	2.39	2.59	1.46	1.71	1.59
ALA	0.61	0.79	1.17	1.86	1.94	2.65	1.24	1.77	1.50
VAL	0.79	0.77	1.58	1.87	2.61	2.72	1.66	1.79	1.72
ILE	0.71	0.90	0.41	2.03	2.24	2.98	1.45	1.97	1.71
LEU	1.42	1.55	2.81	3.47	4.44	5.05	2.89	3.36	3.12
TYR	0.26	0.21	0.66	0.59	0.94	0.83	0.62	0.55	0.58
PHE	0.89	0.92	1.88	2.06	2.81	3.00	1.86	2.00	1.93
HIS	0.53	0.57	1.05	1.30	1.66	1.89	1.08	1.26	1.17
LYS	0.54	0.73	1.03	1.66	1.60	2.33	1.06	1.57	1.31
ARG	0.97	1.39	1.83	3.07	2.98	4.29	1.92	2.92	2.42
PRO	2.32	1.98	4.52	4.27	6.96	6.28	4.60	4.18	4.39
MEAN	1.15	1.06	2.30	2.41	3.61	3.51	2.35	2.32	
MEAN		1.10		2.35		3.56			2.34

Table 10 Apparently Digestible Amino Acids From 4 Wheat Samples 5002 Hard 1B1R;5005 Soft -1B1R: 5011 Hard 1B1R: 5020 Soft -1B1R

TRIAL 6	RATE OF INCLUSION																
AMINO ACIDS	250				500				750				MEAN				
	5002	5005	5011	5020	5002	5005	5011	5020	5002	5005	5011	5020	5002	5005	5011	5020	MEAN
CYS	0.53	0.63	0.57	0.56	0.96	1.30	1.27	1.19	1.74	2.10	1.89	1.79	1.08	1.34	1.24	1.18	1.21
MET	0.35	0.43	0.37	0.40	0.62	1.06	0.81	0.82	1.10	1.32	1.20	1.22	0.69	0.94	0.80	0.81	0.801
ASP	0.94	1.07	0.90	0.98	1.60	2.10	2.19	2.16	3.02	3.75	3.29	3.21	1.85	2.30	2.13	2.11	2.10
THR	0.41	0.51	0.38	0.45	0.74	1.14	1.07	1.10	1.48	2.09	1.61	1.70	0.88	1.25	1.02	1.08	1.06
SER	0.80	1.04	0.77	0.89	1.39	2.09	1.91	2.00	2.59	3.55	2.93	3.00	1.59	2.23	1.87	1.96	1.91
GLU	3.19	2.83	2.31	2.27	5.51	5.48	5.00	4.80	9.23	9.07	7.48	7.01	6.14	5.79	4.93	4.70	5.39
GLY	0.66	0.78	0.60	0.67	1.17	1.55	1.49	1.52	2.19	2.70	2.22	2.22	1.34	1.68	1.44	1.47	1.48
ALA	0.67	0.77	0.63	0.67	1.15	1.48	1.52	1.48	2.12	2.60	2.25	2.18	1.32	1.62	1.47	1.44	1.46
VAL	0.70	0.85	0.66	0.70	1.30	1.74	1.60	1.61	2.40	2.99	2.41	2.36	1.46	1.86	1.56	1.56	1.61
ILE	0.72	0.81	0.73	1.75	1.27	1.61	1.63	1.62	2.28	2.69	2.45	2.41	1.42	1.71	1.60	1.59	1.58
LEU	1.35	1.51	1.29	1.34	2.37	2.99	2.88	2.89	4.24	4.94	4.34	4.29	2.65	3.15	2.84	2.84	2.87
TYR	0.30	0.36	0.30	0.26	0.62	0.76	0.63	0.60	1.21	1.23	1.08	0.90	0.71	0.78	0.67	0.58	0.69
PHE	0.94	0.96	0.84	0.81	1.65	1.92	1.83	1.75	2.95	3.12	2.80	2.61	1.85	2.00	1.82	1.73	1.85
HIS	0.51	0.62	0.48	0.49	0.88	1.21	1.10	1.06	1.65	2.06	1.69	1.55	1.01	1.30	1.09	1.03	1.11
LYS	0.63	0.57	0.54	0.60	1.03	0.94	1.22	1.39	1.84	1.73	1.85	1.83	1.17	1.08	1.21	1.24	1.17
ARG	1.12	1.10	0.58	1.11	1.87	1.94	1.42	2.33	3.29	2.54	2.16	3.42	2.09	1.86	1.39	2.29	1.91
PRO	1.89	1.69	1.11	1.85	3.34	3.27	2.43	3.86	5.91	5.22	3.73	5.73	3.71	3.40	2.42	3.81	3.34
MEAN	0.92	0.97	0.77	0.87	1.62	1.92	1.77	1.89	2.93	3.16	2.67	2.79	1.82	2.02	1.73	1.85	
MEAN				0.88				1.80				2.89					1.86

Table 11 Regression Data for Trial 1 Wheat A

AMINO ACIDS	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFFICIENT APP.	COEFFICIENT TRUE
CYS	-0.149	0.0023	0.999	2.17	2.57	0.847	0.905
MET	-0.072	0.0016	0.999	1.50	1.72	0.871	0.913
ASP	-0.574	0.0050	0.999	4.46	5.92	0.753	0.850
THR	-0.465	0.0029	0.995	2.46	3.35	0.734	0.873
SER	-0.427	0.0045	0.999	4.22	5.14	0.814	0.904
GLU	-0.645	0.0153	0.999	14.70	16.99	0.865	0.903
GLY	-0.340	0.0035	0.999	3.16	4.18	0.756	0.837
ALA	-0.316	0.0035	0.998	3.26	4.15	0.777	0.853
VAL	-0.387	0.0038	0.998	3.37	4.31	0.782	0.872
ILE	0.387	0.0038	0.999	3.22	3.85	0.837	0.905
LEU	-0.410	0.0065	0.999	6.09	7.17	0.850	0.907
TYR	-0.146	0.0024	0.999	2.27	2.69	0.843	0.900
PHE	-0.151	0.0050	0.981	4.82	5.35	0.900	0.928
HIS	-0.165	0.0027	0.999	2.50	3.01	0.832	0.887
LYS	-0.183	0.0028	0.999	2.66	3.37	0.726	0.844
ARG	-0.337	0.0051	0.999	4.81	5.93	0.812	0.868
PRO	-0.959	0.0074	0.983	6.45	8.51	0.758	0.871

Table 12 Regression Data for Trial 1 Wheat B

	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFFICIENT	
AMINO ACIDS						APP.	TRUE
CYS	-0.279	0.0028	0.991	2.55	2.98	0.858	0.951
MET	-0.090	0.0018	0.994	1.67	1.96	0.845	0.898
ASP	-0.830	0.0545	0.968	4.62	6.47	0.715	0.843
THR	-0.797	0.0036	0.750	2.80	3.74	0.750	0.963
SER	-0.712	0.0053	0.982	4.61	5.58	0.826	0.954
GLU	-0.927	0.0099	0.977	9.01	11.92	0.756	0.834
GLY	-0.470	0.0035	0.969	3.00	4.29	0.702	0.809
ALA	-0.399	0.0034	0.972	3.01	4.25	0.709	0.803
VAL	-0.478	0.0037	0.976	3.26	4.54	0.719	0.825
ILE	-0.373	0.0038	0.986	3.38	4.16	0.813	0.903
LEU	-0.563	0.0069	0.989	6.30	7.64	0.825	0.898
TYR	-0.195	0.0027	0.985	2.53	3.10	0.817	0.880
PHE	-0.358	0.0055	0.990	5.13	5.95	0.863	0.923
HIS	-0.229	0.003	0.986	2.63	3.28	0.804	0.874
LYS	-0.315	0.0029	0.982	2.61	3.48	0.750	0.761
ARG	-0.402	0.0052	0.981	4.84	6.36	0.761	0.824
PRO	-1.191	0.0082	0.987	6.96	9.24	0.753	0.882

Table 13 Regression Data for Trial 1 Wheat C

AMINO ACIDS	B	A	R^2	SOLN. X = 100 0	LEVEL g/kg	COEFFICIENT	
						APP.	TRUE
CYS	-0.095	0.0082	0.987	3.14	9.24	0.753	0.882
MET	-0.058	0.00202	0.998	1.97	2.21	0.889	0.915
ASP	-0.359	0.0057	0.988	5.30	7.14	0.742	0.793
THR	-0.366	0.0034	0.990	2.99	4.08	0.734	0.823
SER	-0.315	0.0055	0.994	5.19	6.34	0.818	0.868
GLU	-0.384	0.0144	0.996	14.03	16.83	0.834	0.857
GLY	-0.191	0.0041	0.990	3.91	5.17	0.757	0.794
ALA	-0.164	0.0039	0.991	3.77	4.95	0.762	0.795
VAL	-0.227	0.0046	0.992	4.39	5.62	0.782	0.823
ILE	-0.282	0.0046	0.994	4.32	5.05	0.857	0.911
LEU	-0.271	0.0082	0.997	7.97	9.21	0.865	0.894
TYR	-0.100	0.0030	0.992	2.90	3.39	0.855	0.885
PHE	-0.154	0.0066	0.998	6.43	7.26	0.885	0.907
HIS	-0.100	0.0033	0.996	3.22	3.86	0.835	0.861
LYS	-0.130	0.0030	0.985	2.83	3.68	0.769	0.804
ARG	-0.168	0.0059	0.992	5.70	7.17	0.795	0.818
PRO	-0.455	0.0045	0.969	4.08	6.57	0.621	0.691

Table 14 Regression Data for Trial 2

AMINO ACIDS	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFFICIENT APP.	COEFFICIENT TRUE
CYS	0.151	0.0034	0.997	3.90	5.28	0.680	0.651
MET	-0.140	0.0028	0.999	2.71	2.96	0.915	0.962
ASP	-0.524	0.0094	0.999	8.91	10.46	0.852	0.902
THR	-0.362	0.0050	0.998	4.69	5.63	0.833	0.897
SER	-0.402	0.0083	0.999	7.90	8.77	0.901	0.947
GLU	8.347	0.0085	0.998	16.86	13.90	0.883	0.446
GLY	0.285	0.0054	0.997	5.67	7.07	0.802	0.761
ALA	-0.287	0.0062	0.999	5.94	6.96	0.854	0.896
VAL	0.284	0.0064	1.000	6.72	7.79	0.863	0.827
ILE	-0.287	0.0068	0.999	6.49	7.21	0.900	0.940
LEU	0.195	0.0107	0.999	10.94	12.12	0.903	0.887
PHE	-0.297	0.0089	0.999	8.61	9.32	0.924	0.956
HIS	1.339	0.0030	0.999	4.31	5.13	0.841	0.580
LYS	-0.67	0.0053	0.993	4.60	5.31	0.866	0.992
ARG	-0.624	0.0098	0.999	9.18	10.41	0.882	0.942
PRO	0.804	0.0121	0.926	12.94	11.81	1.096	1.028

Table 15 Regression Data for Trial 3 Brigadier 1

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.093	0.0018	0.999	1.67	2.18	0.764	0.807
MET	-0.007	0.0010	0.998	1.07	1.34	0.795	0.800
THR	-0.212	0.0012	0.997	1.03	2.19	0.469	0.566
ALA	-0.128	0.0022	0.999	2.08	3.22	0.645	0.684
LEU	-0.185	0.0045	0.999	4.36	5.58	0.780	0.813
HIS	-0.072	0.0015	0.999	1.40	2.07	0.676	0.711
LYS	-0.058	0.0016	0.996	1.51	2.52	0.598	0.620
ASP	-0.301	0.0031	0.999	2.85	4.61	0.618	0.683
SER	-0.249	0.0023	0.999	2.07	3.16	0.653	0.732
GLU	-6.233	0.0298	0.972	23.58	23.84	0.989	1.25
GLY	-0.109	0.0021	0.984	2.00	3.42	0.584	0.616
VAL	-0.062	0.0023	0.987	2.22	3.68	0.603	0.610
ILE	-0.107	0.0028	0.999	2.67	3.48	0.767	0.800
TYR	-0.059	0.0006	0.972	0.59	1.11	0.531	0.585
PHE	-0.115	0.0024	0.999	2.37	3.08	0.734	0.772
ARG	-0.113	0.0031	0.999	3.07	4.29	0.694	0.721
PRO	-0.666	0.0095	0.998	8.81	10.21	0.864	0.929

Table 16 Regression Data for Trial 3 Brigadier h

AMINO ACIDS	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFFICIENT APP.	COEFFICIENT TRUE
CYS	-0.118	0.0017	0.994	1.73	2.41	0.716	0.750
MET	-0.081	0.0014	0.999	1.37	1.62	0.842	0.892
THR	-0.442	0.0024	0.999	2.33	2.95	0.656	0.806
ALA	-0.378	0.0029	0.999	1.94	3.64	0.706	0.810
LEU	-0.478	0.006	0.999	5.74	6.97	0.824	0.893
HIS	-0.118	0.0017	0.993	1.58	2.44	0.649	0.698
LYS	-0.279	0.0023	0.999	2.02	2.90	0.697	0.793
ASP	-0.753	0.0044	0.973	4.01	5.43	0.739	0.816
SER	-0.417	0.0040	0.999	3.58	4.62	0.776	0.867
GLU	-0.915	0.0028	0.999	26.80	29.34	0.914	0.945
GLY	-0.418	0.0034	0.997	2.93	4.08	0.720	0.823
VAL	-0.459	0.0037	0.996	3.24	4.35	0.744	0.850
ILE	-0.292	0.0042	0.999	3.88	4.61	0.841	0.904
TYR	-0.168	0.0007	0.979	0.53	1.06	0.505	0.664
PHE	-0.296	0.0037	0.999	3.72	4.18	0.819	0.890
ARG	-0.440	0.0042	0.999	3.77	5.09	0.742	0.829
PRO	-0.702	0.011	0.986	10.25	12.14	0.844	0.902

Table 17 Regression Data for Trial 3 Hussar 1398

AMINO ACIDS	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFF. APP.	COEFF. TRUE
CYS	-0.123	0.0023	0.999	2.22	2.72	0.816	0.862
MET	-0.051	0.0015	0.999	1.40	1.65	0.848	0.879
THR	-0.291	0.0022	0.999	1.93	3.04	0.635	0.731
ALA	-0.239	0.0029	0.998	2.68	3.76	0.711	0.775
LEU	-0.284	0.0062	0.998	5.88	7.17	0.820	0.859
HIS	0.568	0.0013	0.946	1.42	2.50	0.568	0.502
LYS	-0.164	0.0022	0.998	2.06	2.98	0.692	0.747
ASP	-0.379	0.0055	0.998	5.12	6.87	0.746	0.801
SER	-0.243	0.0039	0.998	3.92	4.78	0.770	0.821
GLU	-0.492	0.0277	0.999	27.18	29.66	0.916	0.933
GLY	-0.215	0.0033	0.995	3.10	4.20	0.738	0.789
VAL	-0.288	0.0035	0.998	3.55	4.42	0.737	0.802
ILE	-0.157	0.0034	1.000	3.22	3.98	0.809	0.845
TYR	-0.098	0.0010	0.991	0.88	1.40	0.623	0.693
PHE	-0.175	0.0038	0.998	3.67	4.47	0.822	0.861
ARG	-0.268	0.0044	0.995	3.80	5.40	0.704	0.812
PRO	-0.600	0.0120	0.995	11.16	12.81	0.871	0.918

Table 18 Regression Data for Trial 3 Hussar 1477

AMINO ACIDS	B	A	R^2	SOLN X = 1000	LEVEL g/kg	COEFFICIENT	
						APP.	TRUE
CYS	-0.122	0.0017	0.997	1.54	2.06	0.747	0.806
MET	0.020	0.0010	0.996	0.99	1.32	0.749	0.719
THR	-0.576	0.0017	0.988	1.41	2.45	0.576	0.700
ALA	-0.188	0.0023	0.986	2.14	3.23	0.664	0.722
LEU	-0.179	0.0046	0.997	4.44	5.86	0.758	0.789
HIS	-0.103	0.0014	0.989	1.34	2.02	0.665	0.716
LYS	0.028	0.0014	0.998	1.39	2.62	0.531	0.510
ASP	-0.383	0.0036	0.989	3.18	4.86	0.654	0.733
SER	-0.085	0.0024	0.998	2.28	3.7	0.616	0.639
GLU	-0.508	0.0215	0.999	20.97	23.24	0.902	0.924
GLY	-0.253	0.0027	0.987	2.67	3.56	0.681	0.753
VAL	-0.103	0.0023	0.999	2.18	3.62	0.601	0.630
ILE	-0.199	0.0031	0.963	2.88	3.77	0.764	0.817
TYR	-0.111	0.00068	0.947	0.53	1.07	0.531	0.635
PHE	-0.167	0.0028	0.993	2.58	3.34	0.774	0.824
ARG	-0.103	0.0030	0.995	2.93	4.47	0.657	0.681
PRO	-0.507	0.0074	0.998	6.88	8.72	0.790	0.848

Table 19 Regression Data for Trial 4 Wheat 1

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.062	0.0032	0.950	3.14	3.59	0.877	0.894
MET	-0.002	0.0017	0.944	1.69	1.94	0.875	0.874
ASP	-0.017	0.0050	0.905	4.79	6.40	0.749	0.775
THR	-0.210	0.0027	0.885	2.48	3.50	0.711	0.771
SER	-0.108	0.0041	0.817	4.03	4.94	0.817	0.838
GLU	-0.240	0.0122	0.973	11.99	13.94	0.860	0.878
GLY	-0.003	0.0034	0.904	3.34	4.47	0.749	0.768
ALA	0.076	0.0035	0.909	3.38	4.41	0.767	0.785
VAL	-0.089	0.0038	0.924	3.75	4.82	0.779	0.798
ILE	-0.026	0.0035	0.929	3.43	4.11	0.835	0.841
LEU	-0.042	0.0065	0.941	6.44	7.60	0.848	0.853
TYR	-0.024	0.0017	0.920	1.72	2.21	0.779	0.790
PHE	-0.021	0.0043	0.941	4.27	5.00	0.853	0.857
HIS	-0.277	0.0034	0.978	3.10	3.17	0.978	1.066
LYS	-0.016	0.0024	0.909	2.45	3.21	0.765	0.771
ARG	-0.078	0.0047	0.889	4.74	6.28	0.756	0.743
PRO	-0.018	0.0079	0.960	7.95	8.67	0.917	0.915

Table 20 Regression Data For Trial 4 Wheat 2

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.142	0.0028	0.998	2.63	3.14	0.836	0.882
MET	-0.037	0.0017	0.999	1.65	1.9	0.850	0.869
ASP	-0.300	0.0052	0.998	4.95	6.66	0.743	0.788
THR	-0.355	0.0030	0.999	2.69	3.64	0.739	0.836
SER	-0.219	0.0046	0.999	4.40	5.37	0.819	0.860
GLU	-0.265	0.0120	0.998	11.38	14.19	0.802	0.821
GLY	-0.170	0.0035	0.998	3.35	4.58	0.731	0.769
ALA	0.143	0.0031	0.997	3.24	4.54	0.714	0.681
VAL	-0.219	0.0038	0.999	3.63	4.83	0.751	0.796
ILE	-0.130	0.0036	0.999	3.43	4.19	0.819	0.850
LEU	-0.188	0.0666	0.999	6.38	7.70	0.828	0.853
TYR	-0.049	0.0118	0.998	1.13	1.70	0.667	0.696
PHE	-0.131	0.0037	0.999	3.55	4.39	0.809	0.839
HIS	-0.093	0.0025	0.998	2.36	3.08	0.767	0.797
LYS	-0.056	0.0029	0.998	2.85	3.7	0.770	0.785
ARG	-0.105	0.0049	0.998	4.81	6.34	0.759	0.776
PRO	-0.195	0.0082	0.999	7.98	8.86	0.901	0.923

Table 21 Regression Data for Trial 4 Wheat 3

AMINO ACIDS	B	A	R^2	SOLN. X = 1000	LEVEL g/kg	COEFFICIENT APP.	COEFFICIENT TRUE
CYS	0.268	0.0022	0.999	2.47	3.19	0.775	0.691
MET	0.208	0.0014	0.996	1.60	2.00	0.799	0.695
ASP	0.565	0.0042	0.999	4.81	6.93	0.694	0.613
THR	0.203	0.0023	0.992	2.57	3.81	0.674	0.620
SER	0.483	0.0039	0.998	4.41	5.71	0.773	0.689
GLU	1.350	0.0096	0.998	10.94	14.37	0.762	0.668
GLY	0.402	0.0029	0.999	3.280	4.75	0.691	0.607
ALA	0.437	0.0029	0.999	3.31	4.71	0.704	0.611
VAL	0.400	0.0031	0.999	3.531	5.02	0.704	0.625
ILE	0.398	0.0029	0.999	3.34	4.33	0.772	0.680
LEU	0.734	0.0054	0.998	6.18	7.92	0.780	0.688
TYR	0.209	0.0014	0.999	1.64	2.32	0.709	0.619
PHE	0.487	0.0035	0.998	4.029	5.14	0.784	0.689
HIS	0.628	0.0017	0.969	2.30	3.35	0.685	0.468
LYS	0.382	0.0023	0.999	2.78	3.80	0.733	0.632
ARG	0.716	0.0043	0.999	5.02	6.91	0.728	0.624
PRO	0.921	0.0070	0.996	7.88	9.27	0.851	0.751

Table 22 Regression Data for Trial 4 Wheat 4

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.101	0.0028	0.998	2.71	3.29	0.825	0.855
MET	-0.011	0.0017	0.999	1.67	1.99	0.839	0.844
ASP	-0.154	0.077	0.998	4.74	6.64	0.714	0.738
THR	-0.171	0.0027	0.998	2.54	3.71	0.685	0.731
SER	-0.117	0.0044	0.999	4.31	5.40	0.799	0.820
GLU	-0.115	0.0160	0.999	15.85	18.79	0.843	0.850
GLY	0.096	0.0034	0.987	3.46	4.87	0.711	0.692
ALA	-0.037	0.0034	0.997	3.32	4.58	0.726	0.734
VAL	-0.108	0.0042	0.997	4.12	5.44	0.757	0.776
ILE	-0.063	0.0035	0.999	3.45	4.27	0.807	0.822
LEU	-0.076	0.0068	0.999	6.75	8.19	0.825	0.834
TYR	-0.022	0.0018	0.998	1.73	2.34	0.738	0.748
PHE	-0.046	0.0045	0.999	4.43	5.36	0.826	0.834
HIS	-0.037	0.0027	0.997	2.63	3.41	0.772	0.783
LYS	-0.083	0.0027	0.932	2.60	3.59	0.723	0.747
ARG	0.015	0.0049	0.997	4.90	6.55	0.750	0.746
PRO	-0.093	0.0085	0.999	8.43	9.43	0.894	0.904

Table 23 Regression Data for Trial 5 Cappelle

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.089	0.0022	0.991	2.09	2.75	0.762	0.795
MET	-0.019	0.0015	0.998	1.49	1.85	0.805	0.811
ASP	-0.161	0.0038	0.992	3.77	5.74	0.656	0.656
THR	-0.213	0.0022	0.999	2.03	3.35	0.608	0.672
SER	-0.133	0.0037	0.999	3.66	4.77	0.767	0.767
GLU	-0.556	0.0287	0.999	28.43	31.50	0.903	0.911
GLY	-0.265	0.0034	0.981	3.18	4.39	0.726	0.787
ALA	-0.088	0.0027	0.991	2.57	3.99	0.644	0.666
VAL	-0.167	0.0036	0.994	3.48	4.90	0.711	0.745
ILE	-0.081	0.0031	0.997	2.98	3.95	0.754	0.774
LEU	-0.121	0.0060	0.997	5.90	7.53	0.784	0.800
TYR	-0.063	0.0014	0.992	1.30	1.84	0.708	0.742
PHE	-0.054	0.0038	0.999	3.77	4.74	0.797	0.808
HIS	-0.050	0.0023	0.997	2.21	3.03	0.730	0.747
LYS	-0.007	0.0021	0.998	2.12	3.16	0.672	0.674
ARG	-0.078	0.0040	0.992	3.93	5.70	0.689	0.703
PRO	-0.045	0.0093	0.999	9.25	10.39	0.890	0.895

Table 24 Regression Data for Trial 5 Bezostaya

	B	A	R^2	SOLN. X = 1000	LEVEL g/kg	COEFFICIENT	
AMINO ACIDS						APP.	TRUE
CYS	-0.905	0.0030	0.997	2.88	3.46	0.831	0.858
MET	-0.016	0.0021	0.998	2.09	2.43	0.860	0.867
ASP	-0.238	0.0061	0.994	5.89	7.74	0.767	0.791
THR	-0.273	0.0035	0.991	3.23	4.32	0.749	0.813
SER	-0.263	0.0058	0.998	5.54	6.52	0.851	0.891
GLU	-0.318	0.0148	0.998	14.50	17.08	0.849	0.868
GLY	-0.126	0.0036	0.993	3.56	4.85	0.733	0.759
ALA	-0.089	0.00371	0.992	3.62	4.87	0.745	0.763
VAL	-0.172	0.0039	0.994	3.75	5.01	0.749	0.783
ILE	-0.111	0.0042	0.997	4.05	4.87	0.832	0.854
LEU	-0.138	0.0070	0.997	6.85	8.30	0.826	0.842
TYR	-0.080	0.0013	0.981	1.17	1.74	0.673	0.718
PHE	-0.085	0.0042	0.996	4.07	4.98	0.819	0.836
HIST	-0.061	0.0026	0.996	2.57	3.28	0.784	0.803
LYS	-0.019	0.0032	0.992	3.16	4.11	0.784	0.774
ARG	0.017	0.0058	0.991	5.82	7.37	0.770	0.787
PRO	-0.117	0.0086	0.998	8.47	9.45	0.896	0.908

Table 25 Regression Data for Trial 6 Wheat 5002

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP	TRUE
CYS	-0.139	0.0024	0.972	2.23	2.91	0.788	0.836
MET	-0.061	0.0015	0.973	1.44	1.78	0.807	0.842
ASP	-0.227	0.0041	0.956	3.94	6.04	0.652	0.689
THR	-0.19	0.0021	0.952	2.12	3.28	0.594	0.652
SER	-0.194	0.0036	0.962	3.38	4.26	0.793	0.839
GLU	-0.397	0.0131	0.972	12.68	15.82	0.802	0.827
GLY	0.772	0.0031	0.965	2.87	4.26	0.673	0.717
ALA	-0.132	0.0029	0.963	2.76	4.15	0.667	0.699
VAL	-0.228	0.0034	0.969	3.16	4.51	0.701	0.751
ILE	-0.143	0.0031	0.971	2.98	3.93	0.759	0.795
LEU	-0.231	0.0058	0.972	5.54	7.21	0.769	0.801
TYR	-0.200	0.0018	0.971	1.62	2.12	0.766	0.861
PHE	-0.159	0.0040	0.973	3.85	4.84	0.796	0.829
HIS	-0.127	0.0023	0.963	2.15	2.96	0.729	0.772
LYS	-0.048	0.0024	0.962	2.38	3.49	0.683	0.697
ARG	-0.082	0.0043	0.968	4.27	5.91	0.722	0.736
PRO	-0.313	0.0081	0.975	7.74	9.06	0.855	0.889

Table 26 Regression Data for Trial 6 Wheat 5005

	B	A	R^2	SOLN	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.131	0.0030	0.997	2.81	3.26	0.863	0.904
MET	0.044	0.0018	0.945	1.83	2.04	0.896	0.875
ASP	-0.373	0.0054	0.982	5.32	6.63	0.751	0.808
THR	-0.332	0.0032	0.987	2.83	3.8	0.744	0.832
SER	-0.278	0.0050	0.991	4.73	5.74	0.824	0.872
GLU	-0.448	0.0125	0.992	12.03	14.47	0.831	0.862
GLY	-0.245	0.0038	0.987	3.60	4.72	0.763	0.815
ALA	-0.213	0.0037	0.982	3.44	4.54	0.758	0.805
VAL	-0.284	0.0043	0.991	4.00	5.06	0.791	0.847
ILE	-0.171	0.0038	0.992	3.58	4.31	0.831	0.871
LEU	-0.276	0.0068	0.993	6.57	7.88	0.834	0.869
TYR	-0.078	0.0017	0.997	1.64	2.14	0.770	0.807
PHE	-0.153	0.0043	0.996	4.16	4.94	0.843	0.874
HIS	-0.147	0.0029	0.989	2.74	3.28	0.837	0.882
LYS	-0.090	0.0023	0.957	2.24	3.60	0.623	0.648
ARG	0.423	0.0029	0.989	3.30	6.57	0.502	0.374
PRO	-0.135	0.0071	0.996	6.93	9.03	0.782	0.829

Table 27 Regression Data for Trial 6 Wheat 5011

	B	A	R^2	SOLN.	LEVEL	COEFFICIENT	
AMINO ACIDS				X = 1000	g/kg	APP.	TRUE
CYS	-0.079	0.0026	0.998	2.57	3.09	0.832	0.857
MET	-0.039	0.0017	0.998	1.63	1.92	0.851	0.871
ASP	-0.267	0.0048	0.997	4.52	6.33	0.714	0.756
THR	-0.221	0.0025	0.996	2.26	3.35	0.675	0.741
SER	-0.291	0.0043	0.998	4.32	5.07	0.795	0.852
GLU	-0.241	0.010	0.999	10.10	12.69	0.796	0.815
GLY	-0.176	0.0032	0.996	3.05	4.27	0.715	0.756
ALA	-0.152	0.0032	0.996	3.09	4.27	0.725	0.760
VAL	-0.200	0.0035	0.997	3.32	4.45	0.745	0.790
ILE	-0.118	0.0034	0.999	3.32	4.1	0.810	0.839
LEU	-0.209	0.0061	0.999	5.88	7.25	0.811	0.840
TYR	-0.115	0.115	0.993	1.46	1.89	0.772	0.833
PHE	-0.137	0.0039	0.999	3.78	4.6	0.821	0.851
HIS	-0.119	0.0024	0.999	2.30	3.02	0.763	0.803
LYS	-0.100	0.0026	0.999	2.51	3.49	0.729	0.748
ARG	-0.202	0.0032	0.998	2.97	6.06	0.490	0.524
PRO	-0.191	0.0052	0.999	5.03	0.862	0.584	0.606

Table 28 Regression Data for Trial 6 Wheat 5020

AMINO ACIDS	B	A	R^2	SOLN. X = 1000	LEVEL g/kg	COEFFICIENT APP.	COEFFICIENT TRUE
CYS	-0.055	0.0052	0.999	2.41	3.03	0.798	0.817
MET	-0.055	0.0025	0.999	1.63	1.99	0.820	0.824
ASP	-0.115	0.0045	0.998	4.34	6.48	0.670	0.688
THR	-0.171	0.0025	0.999	2.34	3.59	0.650	0.698
SER	-0.146	0.0042	0.999	4.07	5.37	0.759	0.786
GLU	-0.045	0.0095	0.998	9.43	12.53	0.752	0.756
GLY	-0.073	0.0031	0.996	3.01	4.48	0.673	0.689
ALA	-0.063	0.0030	0.998	2.95	4.35	0.679	0.694
VAL	-0.106	0.0033	0.997	3.22	4.64	0.695	0.718
ILE	-0.063	0.0033	0.999	3.25	4.20	0.775	0.790
LEU	-0.110	0.0059	0.999	5.78	7.45	0.777	0.792
TYR	-0.059	0.0013	0.998	1.22	1.85	0.662	0.694
PHE	-0.405	0.0036	0.725	3.19	4.49	0.711	0.801
HIS	-0.031	0.0021	0.997	2.09	2.96	0.707	0.718
LYS	0.009	0.0025	0.995	2.44	3.58	0.688	0.685
ARG	-0.240	0.0046	0.998	4.60	6.31	0.725	0.734
PRO	-0.062	0.0078	0.999	7.69	9.01	0.854	0.861

Table 29 Content of Amino Acids in Wheat (g/16g N)

	TRIAL ONE			TRIAL THREE			
	A	B	C	B1	BH	H1398	H1477
NITROGEN	17.40	18.35	20.85	15.70	20.00	19.70	14.70
AMINO ACIDS							
CYS	2.36	2.60	2.77	2.22	1.93	2.21	2.24
MET	1.58	1.71	1.70	1.37	1.30	1.34	1.44
ASP	5.44	5.64	5.48	4.70	4.34	5.58	5.29
THR	3.08	3.26	3.13	2.23	2.36	2.47	2.67
SER	4.73	4.87	4.87	3.22	3.70	3.88	4.03
GLU	15.62	10.39	12.92	24.30	23.47	24.09	25.30
GLY	3.84	3.74	3.97	3.49	3.26	3.41	3.54
ALA	3.82	3.71	3.80	3.28	2.91	3.05	3.52
VAL	3.96	3.96	4.31	3.75	3.48	3.59	3.94
ILE	3.54	3.63	3.88	3.55	3.69	3.23	4.10
LEU	6.59	6.66	7.07	6.69	5.58	5.82	6.38
TYR	2.47	2.70	2.60	1.13	0.85	1.14	1.16
PHE	4.92	5.19	5.57	3.14	3.34	3.63	3.64
HIS	2.77	2.86	2.96	2.11	1.95	2.03	2.20
LYS	3.10	3.03	2.82	2.57	2.32	2.42	2.85
ARG	4.96	5.55	5.50	4.37	4.07	4.39	4.87
PRO	7.83	8.06	5.04	10.41	9.71	10.40	9.49

Table 30 Content of Amino Acids in Wheat (g/16g N)

	TRIAL FOUR				TRIAL FIVE	
	1	2	3	4	CAP	BEST
NITROGEN	18.35	18.15	18.85	18.70	19.40	21.80
AMINO ACIDS						
CYS	3.13	2.77	2.71	2.81	2.27	2.54
MET	1.69	1.67	1.70	1.70	1.53	1.78
ASP	5.58	5.87	5.88	5.68	4.73	5.68
THR	3.05	3.21	3.23	3.17	2.76	3.17
SER	4.31	4.73	4.85	4.62	3.93	4.79
GLU	12.15	12.51	12.20	16.08	25.98	12.54
GLY	3.90	4.04	4.03	4.17	3.62	3.56
ALA	3.85	4.00	4.00	3.92	3.29	3.57
VAL	4.20	4.26	4.26	4.65	4.04	3.68
ILE	3.58	3.69	3.68	3.65	3.26	3.57
LEU	6.63	6.79	6.72	7.01	6.21	6.09
TYR	1.93	1.50	1.97	2.00	1.52	1.28
PHE	4.36	3.87	4.36	4.59	3.91	3.66
HIS	2.76	2.72	2.84	2.92	2.50	2.41
LYS	2.80	3.26	3.23	3.07	2.61	3.02
ARG	5.48	5.59	5.87	5.60	4.70	5.41
PRO	7.56	7.81	7.22	8.07	8.57	6.94

Table 31 Content of Amino Acid in Wheat (g/16g N)

TRIAL SIX	5002	5005	5011	5020
NITROGEN	17.40	17.90	18.20	19.00
AMINO ACIDS				
CYS	2.68	2.91	2.72	2.55
MET	1.64	1.82	1.69	1.68
ASP	5.55	5.93	5.56	5.46
THR	3.02	3.40	2.95	3.02
SER	3.92	5.13	4.46	4.52
GLU	14.55	12.93	11.16	10.55
GLY	3.92	4.22	3.75	3.77
ALA	3.82	4.06	3.75	3.66
VAL	4.15	4.52	3.91	3.91
ILE	3.61	3.85	3.60	3.54
LEU	6.63	7.04	6.37	6.27
TYR	1.95	1.91	1.66	1.56
PHE	4.45	4.42	4.04	3.78
HIS	2.72	2.93	2.65	2.49
LYS	3.21	3.22	3.07	3.01
ARG	5.43	5.87	5.33	5.31
PRO	8.33	8.07	7.58	7.59

Table 32 Anova for Data From Trial 1

FACTOR	P	SED
FERT	< 0.001	0.023
AACID	< 0.001	0.055
Lin	< 0.001	0.012
Quad	< 0.001	0.020
FERT.ACID	< 0.001	0.095
FERT.INCL	< 0.001	0.040
FERT Lin	< 0.001	0.020
FERT Quad	< 0.001	0.035
AACID.INCL	< 0.001	0.095
AACID Lin	< 0.001	0.048
AACID Quad	0.797	0.083
FERT.AACID.INCL	< 0.001	0.165
FERT.AACID Lin	< 0.001	0.825
FERT.AACID Quad	0.831	0.143
TIME	0.439	0.019

Table 33 Anova for Data From Trial 2

FACTOR	P	SED
AACID	< 0.001	0.067
INCL	< 0.001	0.029
Lin	< 0.001	0.015
Quad	0.085	0.025
AACID.INCL	< 0.001	0.116
AACID Lin	< 0.001	0.015
AACID Quad	0.912	0.025

Table 34 Anova for Data From Trial 3

FACTOR	P	SED
PROT	< 0.001	0.019
AACID	< 0.001	0.039
INCL	< 0.001	0.016
Lin	< 0.001	0.008
Quad	< 0.001	0.014
PROT.AACID	< 0.001	0.077
PROT.INCL	< 0.001	0.033
PROT Lin	< 0.001	0.016
PROT Quad	0.031	0.028
AACID.INCL	< 0.001	0.067
AACID Lin	< 0.001	0.034
AACID Quad	0.012	0.058
PROT.AACID.INCL	< 0.001	0.135
PROT.AACID Lin	< 0.001	0.067
PROT.AACID Quad	0.999	0.117
TIME	< 0.001	0.013

Table 35 Anova for Data From Trial 3

FACTOR	P	SED
WHEAT	< 0.001	0.033
AACID	< 0.001	0.069
INCL	< 0.001	0.029
Lin	< 0.001	0.014
Quad	< 0.001	0.250
WHEAT.AACID	< 0.001	0.137
WHEAT.INCL	< 0.001	0.058
WHEAT Lin	< 0.001	0.029
WHEAT Quad	< 0.001	0.050
AACID.	< 0.001	0.119
AACID Lin	< 0.001	0.060
AACID Quad	0.992	0.103
WHEAT.AACID.INCL	0.191	0.238
WHEAT.AACID Lin	< 0.001	0.119
WHEAT.AACID Quad	0.999	0.206
TIME	0.004	0.024

Table 36 Anova for Data From Trial 5

FACTOR	P	SED
WHEAT	0.107	0.017
AACID	< 0.001	0.051
INCL	< 0.001	0.021
Lin	< 0.001	0.011
Quad	0.261	0.018
WHEAT.AACID	< 0.001	0.072
WHEAT.INCL	< 0.001	0.030
WHEAT Lin	0.794	0.015
WHEAT Quad	< 0.001	0.026
AACID.INCL	< 0.001	0.088
AACID Lin	< 0.001	0.044
AACID Quad	1.00	0.076
WHEAT.AACID.INCL	< 0.001	0.124
WHEAT.AACID Lin	< 0.001	0.062
WHEAT.AACID Quad	0.647	0.107

Table 37 Anova for Data From Trial 6

FACTOR	P	SED
WHEAT	< 0.001	0.035
AACID	< 0.001	0.073
INCL	< 0.001	0.031
Lin	< 0.001	0.015
Quad	< 0.001	0.027
WHEAT.AACID	< 0.001	0.146
WHEAT.INCL	< 0.001	0.061
WHEAT Lin	0.004	0.031
WHEAT Quad	< 0.001	0.053
AACID.INCL	0.001	0.126
AACID Lin	< 0.001	0.063
AACID Quad	0.986	0.109
WHEAT.AACID.INCL	0.748	0.253
WHEAT.INCL Lin	0.025	0.126
WHEAT.AACID Quad	1.000	0.219
TIME	0.002	0.025