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THE USE OF FIELD BEANS AS A FEED FOR BROILER CHICKENS

By

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ABSTRACT

The chemical composition and physical characteristics of ten different UK grown field bean cultivar samples from the same harvest year were determined. Compositional variation existed between the beans. In the first experiment, diets that included each bean sample at 200 g/kg were used to compare broiler growth performance and determine available energy and nutrient utilisation. Relationships were examined between the nutritional variables measured in broilers and the laboratory characterisation of the field bean samples. Findings showed differences (P<0.05) among the bean cultivar samples for feed efficiency, available energy and amino acid digestibility. A step-wise regression technique indicated that crude protein and ash content of the beans were the explanatory variables that significantly affected the previously unexplained variation in metabolisable energy and amino acid digestibility. The second study tested whether the addition of exogenous xylanase, phytase and protease, alone or in combination could improve the feeding value of beans for broilers. The enzymes had little or no effect on energy and nutrient availability for broilers. The third experiment looked at the use of a tannase-containing enzyme product (that additionally had α-amylase, xylanase, and pectinase activities) on the feeding value of a high tannin field bean cultivar. The enzyme increased (P<0.05) dietary metabolisable energy, nutrient utilisation coefficients and also reduced the weight of the pancreas. The fourth experiment examined the impact of tannase-containing enzyme product on the feeding value of field beans with different tannin contents. The enzyme improved (P<0.001) feed efficiency, energy, nutrient availability and reduced ileal digesta viscosity, despite tannin level in the beans. The fifth study demonstrated that micronising did not improve the nutritional value of beans for broilers. It was concluded that supplementation with the tested tannase containing enzyme mixture can improve the overall energy and nutrient availability of chicken diets, but not field beans specifically.

DECLARATION

This thesis has been composed by the author and is a record of the work which has been done by him on a novel line of research and has not been accepted in any application for any qualification or degree. All sources of information have been illustrated in the texts and displayed in the references. All help offered by others has been mentioned in the acknowledgements.

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PUBLISHED WORK

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LIST OF ABBREVIATIONS

a*	Redness-greenness colour score
AA	Amino acid
ADF	Acid detergent fibre
AID	Apparent ilial digestibility coefficient
AME	Apparent metabolisable energy
AMEn	Nitrogen-corrected apparent metabolisable energy
ANFs	Anti-nutritional factors
ANOVA	Analysis of variance
APD	Apparent protein digestibility
Ara	Arabinose
b*	Yellowness-blueness colour score
BW	Body weight
Ca	Calcium
cP	Centipoise
CP	Crude protein
CT	Condensed tannins
CTD	Coefficient of condensed tannin digestibility
CV	Coefficient of variation
DCP	Dicalcium phosphate
DM	Dry matter
DMD	Coefficient of ileal dry matter digestibility
DMR	Coefficient of total tract dry matter retention
FCR	Feed conversion ratio
FD	Coefficient of total tract fat digestibility
FI	Daily feed intake
Fuc	Fucose
Gal	Galactose
GalA	Galacturonic acid
GE	Gross energy
GIT	Gastrointestinal tract
Glu	Glucose
IFD	Coefficient of ileal fat digestibility
I-NSPs	Insoluble non-starch polysaccharides
L*	Lightness-darkness colour score
Man	Mannose
MB	Maris Bead
ME	Metabolisable energy

NDF	Neutral detergent fibre
NPR	Net protein ratio
NR	Coefficient of total tract nitrogen retention
NSPs	Non-starch polysaccharides
NTPH	Non-tannin phenols
P	Probability
PG	Proventriculus and gizzard
PVPP	Polyvinyl-polypyrrolidone
r	Correlation
r^2	Corrected r^2
Rha	Rhamnose
SAc	Concentration of mucin losses as sialic acid in excreta
SAt	Total excreted mucin losses as sialic acid over 96 hours
SBM	Soybean meal
SE	Standard error of observations
SEM	Standard error of means
SI	Small intestine
S-NSPs	Soluble non-starch polysaccharides
T-	Tannin-free field bean cultivars
T- VC-	Tannin-free and vicine and convicine-low field bean cultivars
T- VC+	Tannin-free and vicine and convicine-high field bean cultivars
T+	Tannin-containing field bean cultivars
T+ VC-	Tannin-containing and vicine and convicine-low field bean cultivars
T+ VC+	Tannin-containing and vicine and convicine-high field bean cultivars
TAAC	Total amino acid content in beans
TD	Coefficient of total tannin digestibility
TGW	Thousand bean grain weight
Ti	Titanium dioxide
TME	True metabolisable energy
T-NSPs	Total non-starch polysaccharides
vc^-	Low vicine and convicine gene
VC-	Low vicine and convicine field bean cultivars
VC+	High vicine and convicine field bean cultivars
WEV	Water extract viscsity
WG	Daily weight gain
WHC	Water holding capacity
Xyl	Xylose
zt2	Zero-tannin gene

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CHAPTER 1: LITERATURE REVIEW

1. 1. General introduction

Soybeans, which are a common source of protein in poultry diets due to having a high level of protein and well balanced profile of amino acids (AAs), are an imported feed ingredient in the UK. From 2002-2015 the average total weight of imported soybeans (as whole seed) to the UK was about 805,577 tonnes per year (866,338 tonnes in 2015) (Statista, 2016). Additionally, a high amount (2,250,000 tonnes in 2015-2016) of soybean meal (SBM) is also imported to the UK (AHDB, 2017). The price of SBM is increasing continuously as a result of rising demand globally, particularly after the prohibition of animal protein inclusion in poultry diets by the European Union (O'Neill *et al.,* 2012). A large amount of the available SBM in the market is produced from genetically modified crops which concerns consumers and is not suitable for organic production (Vicenti *et al.,* 2009). According to Gasparri *et al.* (2013), increasing global demand for SBM caused deforestation of millions of hectars in Southern America, especially over the last half century. This has left a negative impact on the carbon footprint and global environmental changes. Furthermore, recently the European Union has encouraged animal producers to exploit locally grown legumes such as field beans in their diet formulation, aiming for ecological and financial benefits (Fru-Nji *et al.,* 2007). Therefore, the search for locally grown alternative protein sources that can totally or at least partially replace SBM is necessary. This would decrease or even end the dependency of the UK poultry feed industry on imported SBM. High concentrations of proteins in field beans and similarity of their AA profile to that of soybeans, renders them to be considered as a desirable candidate to replace SBM, at least partially, in poultry diet formulation. Field beans could possibly be produced at the required amount locally due to the suitability of the British climate for this crop and variety of cultivars, by which some of them can be sown in autumn and others in spring. According to PGRO (2015), due to breeding, the yield of UK field beans has massively increased. In recent years, it is apparent that field beans are being planted in a wide area of the UK and as a consequence of breeding, the yield of spring UK-grown field beans is now 6.28 tonnes/hectare (PGRO, 2016). The annual UK production of field beans is approximately 540,000 tonnes (Redman, 2015), however, very little of it is employed in UK animal feed formulation and the rest is exported. Nowadays, the demand on producing field beans is increasing and this increase is expected to continue, thus they will be an available feedstuff at a high amount in the UK market. Interestingly, the price of field beans is less than half of that of SBM (£156 *versus* £345/tonne) in the UK.

It has been reported that field beans can be included at 20 to 30% in broiler diets without any adverse effect on performance (Farrell *et al.,* 1999; Nalle, 2009; Usayran *et al.,* 2014). The utilisation of field beans in poultry feed formulation is still limited. This is due to

uncertainty about the chemical composition, which may be highly variable between different cultivars, especially with regards to the type and amount of anti-nutrients that they may contain. This may also result in variation of their energy and nutrient availability for broilers.

Adequate and precise information on the chemical composition and nutritive value of different locally-grown field bean cultivars provides flexibility and constancy to the poultry feed industries, allowing them to involve field beans in their diet formulation, as well as minimising the cost due to overfeeding. This increases the efficiency of the diet formulation. According to Tahir and Pesti (2012), knowing the levels of digestible nutrients in feed ingredients (particulally digestible amino acids) and using suitable values for these, lead to an economically efficient feed formulation. However, there is some available information about the availability of protein, AAs and energy of some field bean cultivars for broilers, but it is not possible to be used as a precise guide for formulation of field bean-containing diets by using other different field bean cultivars that are available in the market. It has been ascertained that the chemical composition and feeding quality of field beans for broilers is variable depending on factors such as genotype, environment and their interaction (Duc *et al.,* 1999; Oomah *et al.,* 2011), breed, sex and age of chickens (Usayran *et al.,* 2014).

There is data of some available studies showing the relation of tannins and vicine and convicine (Lacassagne *et al.,* 1988; Vilariño *et al.,* 2009) content of field beans with their protein and energy availability for broilers, but in these studies only a few cultivars were evaluated. Also there are few studies displaying the relation of field bean non-starch polysaccharides (NSPs) to their energy and nutrient availability for broilers (Lacassagne *et al.,* 1991; Nalle, 2009). All these studies have been conducted outside the UK using a few old field bean cultivars. There is only one study in which the relationship between tannin contents and the availability of protein and AA, of a few new UK field bean cultivars, for broilers has been examined (O'Neill *et al.,* 2012). There is no available published study explaining the variation in energy and nutrient availability from the determined all nutrient, anti-nutrient contents and physical properties of a wide range of UK field bean cultivars. Also, information on the variation in nutrient availability among a wide range of UK field bean varieties is lacking. There is a need for further investigation in the relationship between the compositional profile of different field bean cultivars and their energy and nutrient availability.

There are new techniques available, such as enzyme supplementation and micronising for treating dietary ingredients in order to increase their nutritive quality for poultry. The nutritive value of field beans to a high extent, relates to their chemical composition. The presence of anti-nutritional factors (ANFs) such as tannins, vicine and convicine (Vilariño *et al.,* 2009), NSPs (Lacassagne *et al.,* 1991; Nalle, 2009; Woyengo and Nyachoti, 2012) and phytate reduces the availability of nutrients and energy of field beans for broilers.

Thereby some treatments, such as the addition of enzymes (Cowieson *et al.,* 2003) and heat process such as micronising (McNab and Wilson, 1974) may improve the nutritional quality and decrease the variability between field bean cultivars for broilers.

It has been known that dietary supplementation with exogenous enzymes reduces the anti-nutritive effects and improve the feeding quality of legumes for poultry (Hughes *et al.,* 2002; Cowieson *et al.,* 2003). Also, micronising is another strategy that can eliminate or inactivate anti-nutritive substances from peas, thus improve their nutrient bioavailability for poultry (Igbasan and Guenter, 1996, 1997). Very little is known about the impact of of the commercially available enzymes on the nutritive value of field beans for broilers. The interaction of micronising with different field bean cultivars and enzyme supplementation has also not been investigated.

Nowadays, there are some new cultivars available in the market, which are different from the old cultivars, but there is no information available about their chemical composition and nutrient and energy availability for broilers. Therefore, evaluation of chemical composition and feeding quality of new UK field bean cultivars as raw and treated, by applying new methods such as enzyme supplementation and micronising, for broilers is necessary.

This project had three major objectives:

1. To determine the chemical composition and physical characteristics of ten separate UK field bean cultivar samples.
2. To examine the inherent nutrient and energy availability when these field bean cultivars are fed to broilers, and to find out the relation between these variables and the determined composition of the field bean cultivars.
3. To examine whether inclusion of enzymes and/or micronising improves the nutritive value of field beans for broilers.

1. 2. Chemical composition of field beans

Field beans (*Vicia faba* L. var. *minor*) are spring and autumn-sown crops. Their inclusion in poultry diets has increased due to the rising price of SBM, particularly after prohibition of inclusion of animal sources of protein in poultry diets in Europe (Crépon *et al.,* 2010; O'Neill *et al.,* 2012).

It has been ascertained that the chemical composition and nutritional value of various field bean cultivars is different (Makkar *et al.,* 1997; Duc *et al.,* 1999). The nutritional value of field beans is associated with the levels of their nutrient and anti-nutrient contents, which determine the utilisability of their energy and nutrients by broilers (Vilariño *et al.,* 2009).

The aim of this chapter was to compare the chemical composition, energy and nutrient availability of different field bean cultivars and make a comparison between some of these variables with those of other legume seeds such as SBM and peas, which are used in poultry diet formulation. Also, to outline the possible methods that can be employed for improving the nutritive value of field beans for broilers.

1. 2. 1. Field bean seed structure

Field bean seeds have a similar physical structure to that of other legume seeds, which consists of three main segments; seed-coat, storage cotyledons and embryonic axis (Figure 1.1). The proportion of each part depends on the field bean cultivar.

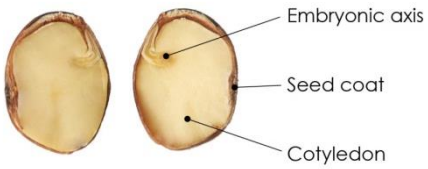


Figure 1. 1. Arthur field bean

1. 2. 1. 1. Seed-coat

The seed-coat is also called the testa or hull, which is the outer shell of a legume seed that covers the storage kernels (cotyledons) and embryonic axis of field beans (Rubio *et al.,* 1992). It has several functions, such as protection of the inner parts of legume seeds from mechanical and environmental factors and it plays an important role in providing nutrients to the embryo sac over the course of embryo progression and seed development, in addition to its effect on marketing attraction. The seed-coat has importance as a passageway for transmission of solutes and also has a vital role in processing of imported metabolites before secretion (Murray, 1987).

The proportion of seed-coat varies from one cultivar to another. The mean proportion of seed-coat to field bean is 11.73%, 12.87%, 14.03% and 14.90% for whole mature spring and winter tannin-free, and spring and winter tannin-containing field bean cultivars, respectively (Duc *et al.,* 1999).

A positive relationship between the darkness of the hull and the tannin contents in legume seeds (for example peas) has been found (Igbasan *et al.,* 1997). Also, Rubio *et al.* (1992) noticed that phytic acid and mineral contents of field beans increase with the darkness of seed-coat. There is a positive correlation between the level of fibres and testa percentage of field beans, as crude fibre forms more than half (535 g/kg DM) of a field bean testa (Marquardt *et al.,* 1975). Also, there is a positive connection between testa proportion and tannin contents in colour-flowering field bean cultivars (Duc *et al.,* 1999).

Total non-starch polysaccharides (T-NSPs) range from 686-863 g/kg DM in field bean testa (Longstaff *et al.,* 1991a; Rubio *et al.,* 1992).

Typically, there is a range of between 54.9-56.4 g/kg DM of CP and 7.1-12.7 g/kg DM of starch content in the hull of field beans (Brufau *et al.,* 1998). However, the concentration of AAs in the field bean seed-coat is relatively close to that of the cotyledon, but both glycine and tyrosine in the hull are about double that in the cotyledon. Also, arginine is much lower than that in kernel. Ash concentration is 25 g/kg DM in field bean testa (Marquardt *et al.,* 1975), and the majority of calcium in faba beans is concentrated in the hull (3.3-3.8 g/kg DM), whereas phosphorus is about 1 g/kg hull DM (Marquardt *et al.,* 1975; Rubio *et al.,* 1992).

Testa, however, is devoid of the lectins, but contains some trypsin inhibitors (Marquardt *et al.,* 1975; Alonso *et al.,* 2000a), and about 3 g/kg DM of phytate (Rubio *et al.,* 1992). All condensed tannins (58-76 g/kg hull DM) and almost all phenolic compounds are concentrated in the testa in field beans (van der Poel *et al.,* 1991; Alonso *et al.,* 2000a).

1. 2. 1. 2. Storage cotyledons

Storage cotyledons are also called kernels, which are the starchy inner part of field bean seeds, which provides the embryo with the required nutrients during the progression period. Cotyledons make up the major proportion of field beans and their proportion varies among cultivars. For instance, the average content for cotyledons and embryonic axis taken together comprises of 88.27, 87.13, 85.97 and 85.10% in spring and winter tannin-free, and spring and winter tannin-containing field bean cultivars, respectively (Duc *et al.,* 1999).

Starch (which ranges from 334 to 471 g/kg DM), and CP (which ranges from 330-406 g/kg DM) are the two main ingredients (Brufau *et al.,* 1998), while crude fibre is low (14 g/kg DM) in field bean cotyledons and embryonic axis together (Marquardt *et al.,* 1975). About 70, 45 and 48% (DM basis) of soluble non-starch polysaccharides (S-NSPs), insoluble non-starch polysaccharides (I-NSPs) and T-NSPs contents of field beans,

correspondingly, are located in their cotyledons and embryonic axis together, and the rest exists in the seed-coat (Nalle *et al.,* 2010b). Faba bean cotyledons and embryonic axis combined contain about 35 g/kg DM of ash (Marquardt *et al.,* 1975), 4.6-6.4 and 0.5 g/kg DM of phosphorus and calcium, respectively (Marquardt *et al.,* 1975; Rubio *et al.,* 1992).
Field bean cotyledons and embryonic axis are free of condensed tannins and contain scarce levels of phenolic compounds (van der Poel *et al.,* 1991; Alonso *et al.,* 2000a), but do contain trypsin, chymotrypsin, α-amylase inhibitors, phytic acid and lectins (Alonso *et al.,* 2000a).

1. 2. 1. 3. Embryonic axis

The embryonic axis is an immature new plant that is in a dormant phase in legume seeds. This part is strongly linked with cotyledons in field beans. When the atmosphere (humidity and temperature) is suitable, the permeability of the seed-coat increases and the activity of hormones increase, thus the embryonic axis starts growing by utilising nutrients from both the seed-coat and the storage cotyledons (Murray, 1987; Smýkal *et al.,* 2014).

1. 2. 2. Nutrients

1. 2. 2. 1. Carbohydrates

1. 2. 2. 1. 1. Starch

Starch, which is a combination of glucans, is the major storage carbohydrate in plantsstored in the cell cytoplasm of plants in the form of insoluble granules consisting of both α-amylose and amylopectin. α-amylose is a straight polymer of several thousands of glucose molecules linked together with α (1→4) linkage (Figure 1. 2). Amylopectin is made up primarily of α (1→4) linked glucose molecules that have α (1→6) branch every twenty-four to thirty glucose molecules (on average) (Figure 1. 3). Amylopectin molecules are composed of up to one million glucose molecules (Voet and Voet, 2004).
Starch is the main source of available energy in poultry diets. However, legume seeds are primarily used as a protein source in poultry diet, but they contain a high level of starch, as well as contributing to dietary available energy content. Starch is predominantly concentrated in cotyledons of legume seeds, and it varies from one bean type to another.
Starch forms about two-thirds of the carbohydrates in mature field bean seeds. Ranges of 407-485 (Makkar *et al.,* 1997), 370-505 (Duc *et al.,* 1999) and 422-451 g/kg DM of starch (Jezierny *et al.,* 2011) have been documented in the literature. The variation in this data may be related to differences in cultivar, environment and the combination of both.
Generally, the concentration of starch in field beans is close to that in peas, but about eight-fold higher than that in SBM (Table 1. 1). There are also differences among cultivars of the same bean type, depending on genotype and environmental factors. The average

of amylose is about 265 and 296 g/kg DM in tannin-free and tannin-containing spring field bean cultivars, respectively (Duc *et al.,* 1999).

Starch content in field beans is significantly connected with genotype (Bjerg *et al.,* 1988; Duc *et al.,* 1999). A mean average of 25 g/kg (on DM basis) of starch in winter field beans above that of spring field bean genotypes has been noted (Duc *et al.,* 1999). Moreover, an average of 17 (Duc *et al.,* 1999) and 20 g/kg DM (Grosjean *et al.,* 2001) of starch in tannin-free (T-) field beans over that in tannin-containing (T+) cultivars have also been noticed.

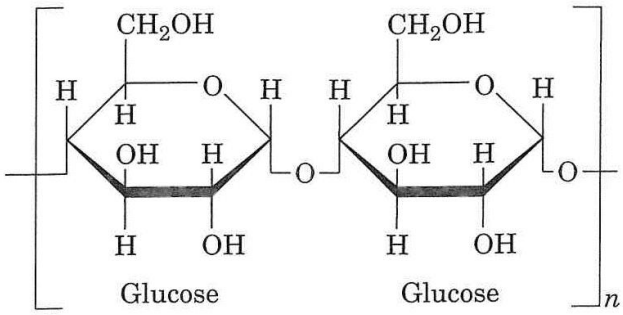


Figure 1. 2. Structure of α-amylose

Adapted from Voet and Voet (2004).

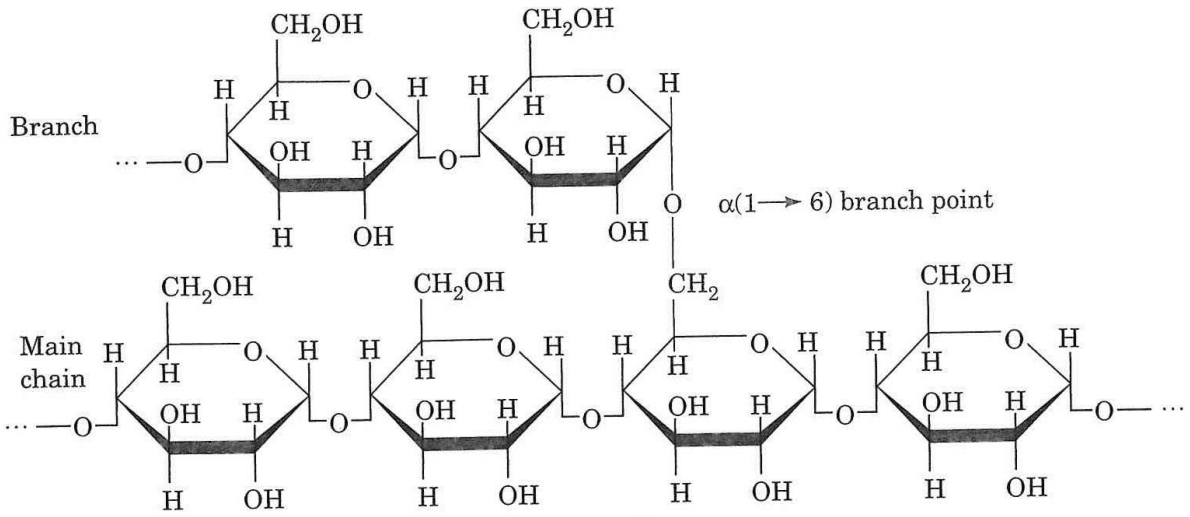


Figure 1. 3. Structure of amylopectin

Adapted from Voet and Voet (2004).

Table 1. 1. Chemical composition of soybean meal, field bean, peas and the range of variation in field beans (g/kg DM)

Parameters	Soybean meal[5]	Field beans[3]	Range in field beans	Peas[3]
Crude protein	529.9	307.8	250.0-337.0[4,3]	248.6
Lipids	18.6	15.1	11.0-47.0[1]	19.9
Ash	75.6	38.0	30.0-55.0[1]	32.7
Starch	50.6[3]	438.0	370.0-505.0[1]	504.5
S-NSPs	17.9	19.6[2]	17.4-21.6[2]	19.2[2]
I-NSPs	192	195[2]	182-218[2]	141[2]
T-NSPs	209.6	214.7[2]	199.0-240.0[2]	160.2[2]

Adapted from [1]Duc *et al.* (1999); [2]Nalle (2009); [3]Jezierny *et al.* (2011); [4]O'Neill *et al.* (2012); [5]Ravindran *et al*. (2014); S-NSPs, soluble non-starch polysaccharides; I-NSPs, insoluble non-starch polysaccharides; T-NSPs, total non-starch polysaccharides.

1. 2. 2. 1. 2. Non-starch polysaccharides

Non-starch polysaccharides are carbohydrates, which have a structural function in legume seeds (Igbasan *et al.*, 1997). They are classified into I-NSPs and S-NSPs.

The concentration of S-NSPs in all field beans, peas and SBM is similar. Also, the amount of I-NSPs in both field beans and SBM is almost the same, which is dramatically higher than that in peas (Table 1. 1).

The NSPs content and profiles are different from one legume type to another and differ among cultivars of the same legume, depending on factors such as environment, genetics and their combination. Perez-Maldonado *et al.* (1999) stated that field bean seeds contain more I-NSPs, S-NSPs and total uronic acid than peas.

Genotype has a considerable effect on NSP concentration in field beans, as zero-tannin (*zt*2) genes reduce seed NSP contents (Bjerg *et al.,* 1988; Duc *et al.,* 1999). White-flowering field beans contain significantly lower NSPs than those in colour-flowering cultivars, but higher than those in SBM (Makkar *et al*., 1997; Duc *et al.,* 1999). In comparison with winter genotypes, spring ones contain a significantly lower level of NSPs (Eden, 1968; Duc *et al.,* 1999). Furthermore, in spring grown field bean genotypes, T- genotypes contain significantly less NSPs than T+ ones (Duc *et al.,* 1999).

Various levels of NSPs in faba beans have been documented previously. Adamidou *et al.* (2011) found 76 and 161 g/kg DM of S-NSPs and I-NSPs in a field bean cultivar, respectively. Whereas, Nalle (2009) reported a range of 17.4-21.6 g S-NSPs and 182-218 g I-NSPs per kg DM among three different New Zealand field bean cultivars.

Glucose, uronic acid, arabinose, xylose, mannose in both insoluble and soluble patterns, and also galactose in an insoluble pattern are the main constituent sugars of field bean NSPs (Perez-Maldonado *et al.,* 1999).

The constituent parts per kg DM of field beans are as follows: 119.7 g T-NSPs; 68.52% is I-NSPs; 28.32% is total uronic acids; 3.17% is S-NSPs including constituent sugars 28.34% glucose; 28.34% uronic acid; 23.92% arabinose; 13.80% xylose; 4.70% mannose and 0.73% galactose (Perez-Maldonado *et al.,* 1999). Moreover, it has been found that the majority of arabinose sugar in field bean NSPs is in the form of arabinose-containing pectin materials in the cell walls of the kernels (Selvendran, 1984; Rubio *et al.,* 1992).

Nalle *et al.* (2010b) reported that about a third of S-NSPs are located in the seed-coat and two thirds in the cotyledons and embryonic axis of field beans and peas. Whereas, about half of their I-NSP contents are located in their seed-coats and the rest is concentrated in their cotyledons and embryonic axis. The NSP profile of the seed-coat and the cotyledons of field beans have been studied separately by Longstaff *et al.* (1991a) and Rubio *et al.* (1992). According to Rubio *et al.* (1992), T-NSP, glucose, uronic acid, xylose, galactose and arabinose accounts to 106 *versus* 686, 27 *versus* 450, 30 *versus* 112, 5.5 *versus* 71, 18 *versus* 23 and 23 *versus* 17 g/kg DM in cotyledons and testa of field beans (respectively).

1. 2. 2. 2. Proteins

Proteins are organic compounds which comprise of a single or more polypeptide chains and are the main source of AAs in broiler diets (Voet and Voet, 2004). Legume seeds are considered a good source of vegetable protein, which is found concentrated in their cotyledons. There is a broad range of CP proportion among different sorts of legume seeds. Overall, field beans contain a slightly higher amount of CP than peas, but contain about 200 g/kg DM lower than that of SBM (Table 1. 1). High variances are seen among cultivars of the same legume type (Table 1. 1), depending on the factors of genotype, environment and the combination of the two (Matthews and Arthur, 1985; Igbasan *et al.,* 1997; Harmankaya *et al.,* 2010). The type of soil and nitrogen application also reflects in CP content of the legume seeds (Igbasan *et al.,* 1996).

There is a high negative relationship between protein and starch in field beans (Duc *et al.,* 1999). Genotype also has a considerable influence on CP content in field beans (Bjerg *et al.,* 1988; Duc *et al.,* 1999). The *zt*2 gene causes a remarkable increase in CP and a significant reduction of the testa proportion, fibre and tannin contents in field beans (Duc *et al.,* 1999). Furthermore, a higher concentration of CP in spring sown field bean genotypes than that in winter genotypes has been observed (Eden, 1968; Bjerg *et al.,* 1988; Duc *et al.,* 1999). An amount of 49 (Eden, 1968) and 23 g/kg DM (Duc *et al.,* 1999) of CP in spring genotypes above winter genotypes has been documented. Also, the concentration of CP in spring-grown tannin-free field bean genotypes is about 10, 19 and 35 g/kg DM above spring-grown tannin-containing, winter-grown tannin-free and winter-grown tannin-containing genotypes (Duc *et al.,* 1999). Compared to spring genotypes, a

lower level of CP content in winter field beans is compensated by higher fibre rather than starch (Duc *et al.,* 1999).

Larralde and MartÍnez (1991) stated that field bean CP comprises of 60% globulins, 20% albumin and 15% glutelin and prolamins. There is a range of 21.6-26.4 g/kg DM soluble protein in colour-flowering, while it ranges from 18.7-21.4 g/kg DM in white-flowering field bean cultivars (Makkar *et al.,* 1997).

Amino acids are considered as basic units of peptides and proteins. They cannot be present as a single or a free AA in feedstuffs, but they are linked together with the aid of peptide bonds to form dipeptides, tripeptides, oligopeptides and polypeptides (Voet and Voet, 2004).

Generally, there are twenty AAs, which are categorised into two groups: indispensable and dispensable AAs. Indispensable AAs include lysine, methionine, histidine, arginine, phenylalanine, isoleucine, leucine, threonine, tryptophan and valine, which cannot be formed in the body of birds, and thereby they must be supplied in the diet. The rest of the amino acids are categorised as dispensable AAs (Voet and Voet, 2004), which can be created in the body of birds. Thereby, the deficiency of dispensable AAs in poultry diets does not lead to any significant problems (Blair, 2008).

The mean average AA profiles of SBM, field bean and peas are presented in Table 1. 2. The AA profile of legume seeds vary from one legume type to another, but there is insignificant or very small differences among cultivars of the same legume type, for example SBM (Ravindran *et al.,* 2014) and peas (Igbasan *et al.*, 1997; Jezierny *et al.,* 2011) produced in the same or even in different regions. All legume seeds have a deficiency of threonine. Peas, field bean and SBM are rich with lysine. However, SBM contains reasonable levels of tryptophan, but a shortage of this AA can clearly be noticed in field beans and peas (Table 1. 2).

There is a large variation in the concentration of AAs between field bean cultivars grown in different regions (Table 1. 3). A high positive relation between the concentrations of AA and CP in field beans (Mossé and Baudet, 1977; Bjerg *et al.,* 1988; Duc *et al.,* 1999) and peas (Bastianelli *et al.,* 1998) have been documented. Spring-sown field beans, especially T- cultivars, have a slightly higher level of AA than winter-grown cultivars (Duc *et al.,* 1999).

Makkar *et al.* (1997) found a relatively broad range of differences in arginine, histidine, lysine, phenylalanine, valine, glutamic acid and proline among twelve different white and colour-flowering field bean cultivars. Whereas, Duc *et al.* (1999) reported very small differences in AA content of 12 different field bean genotypes, regardless of their sowing season, tannin and vicine and convicine content levels. These researchers found a low positive correlation between CP content and glutamine, methionine, phenylalanine and histidine in field beans, while the relationship between the concentration of CP and other AAs were significantly positive.

Table 1. 2. Comparison of amino acid content (g/kg CP) of soybean meal, peas and field beans

Amino acid	Soybean meal[4]	Field beans[1]	Peas[3]
Indispensable amino acids			
Arginine	74.0	91.4	90.1
Histidine	28.2	25.7	24.5
Isoleucine	46.9	43.2	41.4
Leucine	76.0	47.6	70.8
Lysine	59.3	65.3	72.0
Methinonine	14.6	8.6	9.3
Phenylalanine	52.5	41.9	47.1
Threonine	38.7	38.6	36.2
Tryptophan	13.5[3]	8.9	9.3
Valine	51.2	50.2	46.7
Dispensable amino acids			
Alanine	41.3	42.6	42.2
Aspartic Acid	116.6	105.9	115.0
Cysteine	15.2	12.2	14.1
Glutamic acid	179.2	147.3	165.3
Glycine	40.6	43.9	42.6
Proline	51.9	40.6	41.0
Serine	46.4	48.2	45.5
Tyrosine	36.6	22.1	29.2[2]

Adapted from [1]Duc *et al.* (1999); [2]Diaz *et al.* (2006); [3]Jezierny *et al.* (2011); [4]Ravindran *et al*. (2014).

Compared to the poultry requirements for AAs, the CP of field beans is high in lysine, but low in methionine, cysteine and tryptophan (Palmer and Thompson, 1975; Mossé and Baudet, 1977; Bjerg *et al.,* 1988). Employing field beans and peas as a main protein source in poultry diets can therefore lead to methionine, cysteine, threonine and tryptophan deficiency, especially in maize-bean based diets.

From this review it appears that field beans have a higher level of lysine than SBM, but are lower than that of peas. The low concentration of lysine (59.3 g/kg CP) in SBM compared to that (65.3 g/kg CP) in field beans is probably due to denaturation by the prolonged or elevated heating in SBM processing (Mutia *et al.,* 1994; Wiryawan and Dingle, 1999). Soyabean meal contains about 41.1% higher methionine than that found in field beans. There is the same amount of threonine in both field beans and SBM, which is slightly higher than that in peas. Field beans and peas contain similar amounts of histidine, which is slightly lower than that found in SBM. Field beans are the richest bean in arginine concentration, followed by peas. Soybean meal has the highest glutamic acid and tyrosine levels, followed by peas and finally field beans. Generally, the highest aspartic acid, methionine, phenylalanine, proline and tryptophan contents are in SBM,

followed by peas and then field beans, but alanine, isoleucine and valine are higher in field beans than peas (Table 1. 3).

Table 1. 3. Range and comparison of the mean of crude protein (g/kg DM) and amino acids (g/kg CP) in field bean cultivars produced from different locations

	Range	Germany[1]	France[2]	Germany[3]	UK[4]	Egypt[5]
Crude protein	250-337	275	303	308	267	277
Amino acid						
Indispensable amino acids						
Arginine	76.3-121.0	106.2	91.4	94.2	86.3	111.2
Histidine	24.6-41.5	32.7	25.7	25.7	26.3	28.1
Isoleucine	32.9-53.4	40.0	43.2	40.0	41.3	44.9
Leucine	67.8-82.7	76.1	74.6	71.5	73.7	76.4
Lysine	60.2-85.6	72.5	65.3	61.7	65.3	71.4
Methionine	6.3-11.0	9.3	8.6	6.5	19.5 [a]	8.7
Phenylalanine	35.8-81.4	44.4	41.9	41.9	50.2	40.6
Threonine	32.9-43.9	40.2	38.6	34.4	35.0	37.8
Tryptophan	7.5-10.6	-	8.9	8.4	8.6	-
Valine	37.5-56.4	47.3	50.2	44.2	46.1	50.1
Dispensable amino acids						
Alanine	34.3-49.1	40.6	42.6	39.6	-	45.3
Aspartic acid	96.7-124.4	103.0	105.9	107.2	-	118.6
Cysteine	7.1-14.2	12.8	12.2	12.0	-	7.4
Glutamic acid	142.0-198.0	157.4	174.3	162.1	-	174.5
Glycine	40.2-52.0	45.8	43.9	41.3	-	45.9
Proline	31.1-62.9	54.1	40.6	39.6	-	44.5
Serine	41.6-55.3	51.7	48.2	46.8	-	48.2
Tyrosine	16.4-46.0	39.5	22.1	-	-	30.3

Adapted from [1]Makkar *et al.* (1997); [2]Duc *et al.* (1999); [3]Jezierny *et al.* (2011); [4]O'Neill *et al.* (2012); [5]Usayran *et al.* (2014); [a]Methionine+cysteine; -, not determined.

1. 2. 2. 3. Lipids and fatty acids

Lipids are organic materials that are completely soluble in organic solvents but only frugally soluble in water. They consist of smaller units that are called fatty acids, which are carboxylic acids that have long-chain hydrocarbon side groups (Voet and Voet, 2004). There is a difference between the lipid content of the different types of legume seeds. In general, field beans contain slightly lower lipids than peas and SBM (Table 1. 1). Concentrations of 11-47 (Duc *et al.,* 1999), 13-17 (Jezierny *et al.,* 2011) and 14-25 g/kg DM of lipids have been reported among many field bean cultivars. Generally, both T+ and T- field bean cultivars contain similar amounts of lipids (Makkar *et al.,* 1997; Duc *et al.,* 1999; Grosjean *et al.,* 2001).

Winter field bean genotypes contain slightly more lipids than spring ones. Compared with spring tannin-containing field bean genotypes (18 g/kg DM), winter tannin-containing field bean genotypes contain a slightly higher amount (24 g/kg DM) of lipids (Duc *et al.,* 1999).
Regarding fatty acids, linolenic acid accounts for 5.8 and 4.7 g/kg DM in field bean and peas, respectively, while it accounts for 27.9 g/kg DM in SBM (Blair, 2008). However, there is a relatively low concentration of total lipids in field beans, but unsaturated fatty acids, such as linoleic (46-54% of lipids) and oleic (24-37% of lipids) acids exist at a high proportion of their lipids (Duc *et al.,* 1999).

1. 2. 2. 4. Minerals and vitamins

Legume seeds contain both major and trace minerals at dissimilar levels, depending on the legume type (Table 1. 4), cultivar (Table 1. 5), soil and the area where they are produced (Ravindran *et al.,* 2014; Zelalem and Chandravanshi, 2014).
Significant differences in the mineral concentrations amongst various cultivars of SBM (Ravindran *et al.,* 2014) and peas (Harmankaya *et al.,* 2010) produced in the same or different regions have been documented. There is similar levels of minerals among winter and spring-grown (Eden, 1968), and white and colour-flowering field bean cultivars (Makkar *et al.,* 1997) (Table 1. 5).
All field bean cultivars have a high deficiency of sodium, manganese and calcium, and some iron limitations, whereas they contain a high level of potassium and the required level of phosphorus, magnesium, copper and zinc with regards to broiler requirements.
Field beans are free of cobalamin and have a very low content of pantothenic acid, biotin and vitamin E, whereas the level of thiamine, pyridoxine, choline and folacin in field beans is at the standard required level for broilers. However, field beans contain lower levels of all minerals and vitamins compared to SBM but they are richer than peas in all minerals and most vitamins (Blair, 2008) (Table 1. 4).

Table 1. 4. Mineral and vitamin contents of soybean meal, field beans and peas

Item	Soybean meal	Field bean	Peas
Calcium (g/kg DM)	4.21[5]	1.56[1]	0.78[2]
Sodium (g/kg DM)	0.12[5]	0.08[1]	<0.01[4]
Magnesium (g/kg DM)	3.85[5]	1.46[1]	1.53[2]
Phosphorus (g/kg DM)	7.46[5]	5.90[1]	4.90[2]
Potassium (g/kg DM)	25.45[5]	13.66[1]	11.53[2]
Copper (mg/kg DM)	16.48[5]	12.49[1]	7.00[2]
Iron (mg/kg DM)	132.77[5]	64.10[1]	59.00[2]
Manganese (mg/kg DM)	56.98[5]	16.37[1]	13.00[2]
Zinc (mg/kg DM)	58.41[5]	51.42[1]	32.00[2]
Vitamins			
Vitamin E (IU/kg)	7.0[3]	5.0[3]	4.0[3]
Biotin (mg/kg)	0.33[3]	0.09[3]	0.15[3]
Choline (mg/kg)	2623[3]	1670[3]	547[3]
Folacin (mg/kg)	6.4[3]	4.2[3]	0.2[3]
Niacin (mg/kg)	31.0[3]	26.0[3]	31.0[3]
Pantothenic acid (mg/kg)	14.3[3]	3.0[3]	18.7[3]
Pyridoxine (mg/kg)	5.5[3]	3.7[3]	1.0[3]
Riboflavin (mg/kg)	3.4[3]	2.9[3]	1.8[3]
Thiamine (mg/kg)	3.9[3]	5.5[3]	4.6[3]

Adapted from [1]Makkar *et al.* (1997); [2]Wang and Daun (2004); [3]Blair (2008); [4]Nalle (2009); [5]Ravindran *et al.* (2014).

Table 1. 5. Comparison of the mean of mineral contents (Dry matter basis) in spring and winter, colour and white-flowering field bean cultivars

Minerals	Spring[1]	Winter[1]	Colour-flowering[2]	White-flowering[2]
Calsium (g/kg)	1.60	1.90	1.34	1.78
Phosphorus (g/kg)	6.60	6.80	5.88	5.91
Magnesium (g/kg)	1.30	1.30	1.59	1.33
Sodium (g/kg)	<0.100	0.200	0.052	0.117
Potasium (g/kg)	11.70	12.20	13.45	13.88
Copper (mg/kg)	-	-	12.41	12.56
Manganese (mg/kg)	14.00	14.00	16.48	16.26
Zinc (mg/kg)	-	-	52.94	49.90
Iron (mg/kg)	-	-	67.21	64.32
Aluminium (mg/kg)	-	-	12.77	11.46
Sulphur (g/kg)	-	-	1.78	1.93
Boron (mg/kg)	-	-	10.18	11.27

Adapted from [1]Eden (1968); [2]Makkar *et al.* (1997); -, not determined.

1. 2. 3. Anti-nutrients

1. 2. 3. 1. Tannins

Tannins are polyphenolic compounds which are classified into two main types; condensed and hydrolysable tannins, which have different chemical structures and biological influences (Makkar *et al.,* 2007; Brief, 2013). Hydrolysable tannins comprise of phenolic carboxylic acids cohering to a central core of carbohydrate with the aid of ester linkages (Figure 1. 4). They comprise of the ester compounds of galic acid and ellagic acid with a sugar, which is usually glucose, and they are hydrolysed by enzymes and acids into monomeric compounds (Brief, 2013). Condensed tannins, which are called proanthocyanidins or polyflavanoids as well, consist of a polyhydroxylflavonol polymer group, with carbon to carbon bonds between their sub units (Brief, 2013). They are composed of no less than two flavan-3-ols, such as catechin, epicatechin, or the equivalent gallocatechin (Figure 1. 5) (Makkar *et al.,* 2007).

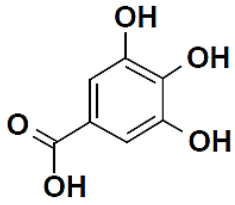
Condensed tannins are considered as the main ANF in colour-flowering field bean cultivars (Cabrera and Martin, 1986; Makkar *et al.,* 1997). These ANFs have negative effects on broiler performance and bioavailability of nutrients such as proteins (Grosjean *et al.,* 1995; Vilariño *et al.,* 2009), AAs (Marquardt and Ward, 1979; Ortiz *et al.,* 1993; Woyengo and Nyachoti, 2012), starch (Lacassagne *et al.,* 1991) and minerals (Marquardt and Ward, 1979) for broilers. Results of *in vitro* and *in vivo* (broiler feeding experiments) studies have shown that tannins inactivate digestive enzymes due to the creation of tannin-enzyme complexes (Longstaff and McNab, 1991a, b; Makkar *et al.,* 1997). Longstaff and McNab (1991a, b) found that the most affected enzymes (in order of high to low) are proteases and amylase, thereby affecting the digestibility of protein and starch in the same order. Field bean tannins also cause histological changes in the ileal mucosa (atrophy and shortening of villi with distortion of their architecture) and liver (hydropic degeneration of hepatocytes) of chicks and rats (Ortiz *et al*., 1994).

Most field bean tannins are located in the seed-coats. Luo and Xie (2013) reported a reduction of 59.2 and 67.3% of tannins in green and white field bean cultivars, respectively, with dehulling. Also, van der Poel *et al.* (1991) found that there is a possibility of removing all condensed tannins in field beans by dehulling.

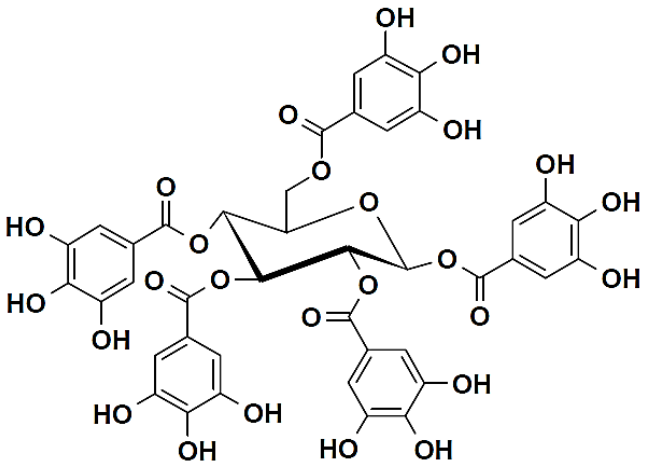
However, some tannin free field bean and pea cultivars have been produced by plant breeders, but most available cultivars of legume seeds that are included in poultry diet (such as soybean, field bean and peas) contain some amount of tannins (Table 1. 6). The levels of both condensed and hydrolysable tannins widely vary depending on legume type. Also, the tannin concentration in legume seeds could be influenced by cultivar, growing location (Oomah *et al.,* 2011) climate, soil condition (Griffiths, 1981; Mansfield *et al.,* 1999) and the duration and condition of storage (Nasar-Abbas *et al.,* 2008, 2009).

Tannins can be removed from field beans by mutation of the *zt*2 gene (Picard, 1976; Duc *et al.,* 1999).

1- Gallotannins

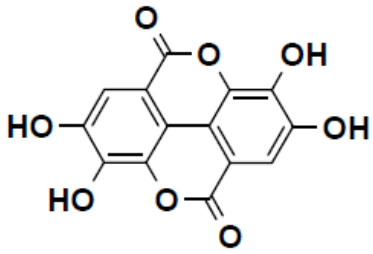


Gallic acid

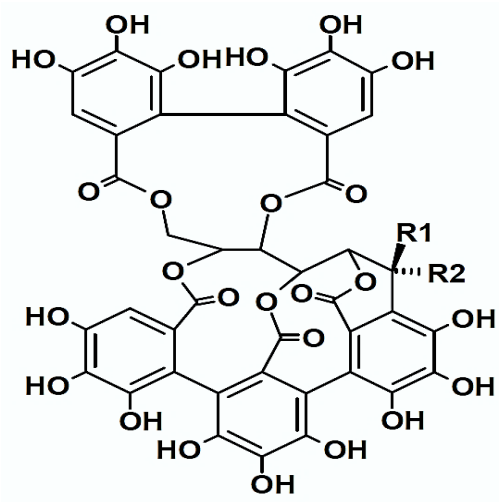


Pentagalloyl glucose (β-1,2,3,4,6-Pentagalloyl-*O*-D-Glucopyranose)

2- Ellagitannins



Ellagic acid



R1=H, R2=OH, Castalagin; R1=OH, R2=H, Vescalagin

Figure 1. 4. Structure of hydrolysable tannins

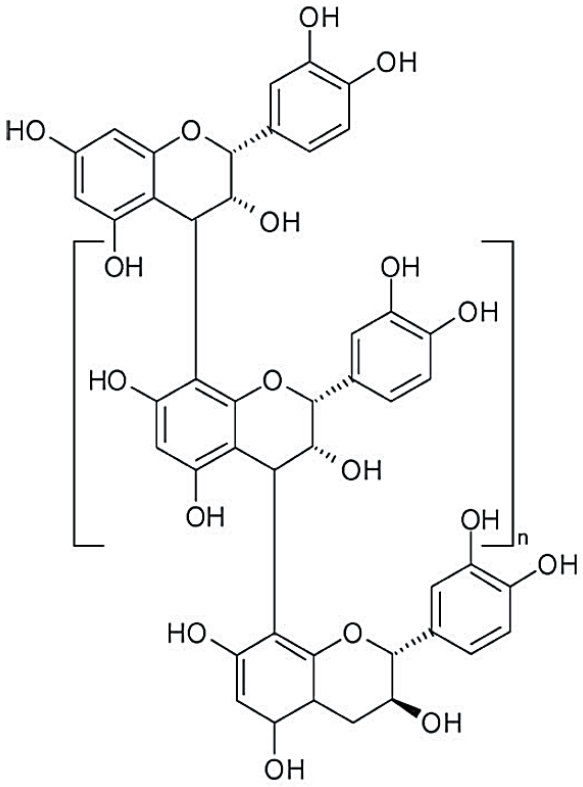
Adapted from Hagerman (2002).

Tannin concentration in legume seeds has a relationship with the seed-coat color; pale-colour seeds have very low tannin concentration and its content increases with the darkness of the seed-coat to the highest level in dark brown seeds (Lindgren, 1975). For example, yellow and green pea cultivars contain less than 1.0 mg/g DM of tannins, but brown coloured pea cultivars contain 11.5-41.0 mg/g DM (Igbasan *et al.,* 1997).

Cabrera and Martin (1986), who studied many lines of field beans, found that tannin content was more related to flower colour than to seed-coat colour in field beans. No more than 2 mg/g DM of tannins (as tannic acid equivalents) are found in white-flowered field bean cultivars, while the concentration is about 10-21 mg/g DM for colour-flowered cultivars (Table 1. 7) (Duc *et al.,* 1995; Makkar *et al.,* 1997). In addition, intermediate concentrations of tannins (as tannic acid) in brown and green-seeded field bean genotypes, with a lower amount in brown than green-colour testas, have been noted (Duc *et al.,* 1995).

It has been reported several times that white-flowering field bean cultivars are free of condensed tannins (Duc *et al.,* 1995; Makkar *et al.,* 1997; Duc *et al.,* 1999), while, their concentration is high in colour-flowering cultivars (Cabrera and Martin, 1986; Makkar *et al.,* 1997).

While condensed tannins (as leucocyanidin equivalents) have not been found in SBM, a mean average of 26.2 g/kg DM has been reported in colour-flowering field bean cultivars. Tannins (as tannic acid equivalent) have, however, been found in SBM and both white-flowering and colour-flowering field bean cultivars (Table 1. 7) (Makkar *et al.,* 1997).



Sorghum procyanidin

Epicatechin-[(4β → 8)-epicatechin]$_{15}$– (4β → 8)-catechin

Figure 1. 5. Structure of condensed tannins

Adapted from Makkar *et al.* (2007).

Table 1. 6. Concentration of some anti-nutritional factors in soybean meal, field bean and peas

Anti-nutritional factor	Soybean meal	Field beans	Peas
Tannins (mg/g DM)	0.39[3]	7.12[1]	0.14[3]
Condensed tannins (mg/g DM)	0.0[4]	13.1[1]	0.0[4]
Phytic acid (mg/g DM)	20.8[5]	15.8[1]	12.2[5]
Trypsin inhibitor activity(mg/g CP)	5.80[4]	1.53[4]	2.97[4]
Saponins (mg/g DM)	49.4[1a]	25.0[1a]	1.8[2]

Adapted from [1]Makkar *et al*. (1997); [2]Allen (1998); [3]Diaz *et al.* (2006); [4]Jezierny *et al.* (2011); [5]Saastamoinen *et al.* (2013); [a]As diosgenin equivalent.

Table 1. 7. Comparison of the range and means of anti-nutrient contents between white (W-F) and colour-flowering (C-F) field bean cultivars (On dry matter basis) produced from different locations

Anti-nutrient	Range in W-F	Range in C-F	W-F Germany[1]	C-F Germany[1]	W-F France[2]	C-F France[2]
Tannins (mg/g)	0.04-2.00[a]	10.30-21.00[a]	0.10[a]	14.10[a]	2.00[a]	11.90[a]
Condensed tannins (mg/g)	0.0[b]	15.7-35.4[b]	0.0[b]	26.2[b]	0.1[d]	6.6[d]
Phytate (mg/g)	11.6-19.4	13.4-20.0	15.0	16.6	-	-
Phytic phosphorus (%)	49.6-82.0	45.3-81.9	70.9	79.2	58.5	55.8
Trypsin inhibitor activity (mg/g)	0.3-5.3	0.1-5.1	3.1	1.9	2.9	2.9
Lectins (mg/g)	0.5-1.8	0.6-1.9	-	-	1.1	1.3
Saponins (mg/g)	16.9-20.1	25.5-39.3	18.3[c]	31.7[c]	-	-
Vicine (mg/g)	0.5-10.4	0.2-10.1	6.8[3]	4.9[3]	6.0	6.0
Convicine (mg/g)	0.1-3.2	0.0-4.3	3.0[3]	2.6[3]	1.6	2.3

Adapted from [1]Makkar *et al.* (1997); [2]Duc *et al.* (1999); [3]Jezierny *et al.* (2011); -, not determined; [a]As tannic acid equivalents; [b]As leucocyanidin equivalents; [c]As diosgenin equivalents; [d]As catechin equivalents.

1. 2. 3. 2. Phytic acid

Phytic acid is the hexaphosphate form of inositol (1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate *myo*-inositol). It is mainly located in the cotyledons and embryonic axis of legume seeds (Reddy and Salunkhe, 1981; Thompson, 1987; Fawzy and Mahmoud, 2003). Rubio *et al.* (1992) detected that phytate is present in both testa and cotyledons of field beans.

Phytic acid can form binary and ternary complexes such as phytic acid-divalent mineral cations (such as Mg^{+2}, Fe^{+2}, Zn^{+2} and Ca^{+2}), phytate-protein, protein-phytate-protein, phytate-mineral-protein and starch-phytate-protein complexes in the intestine of animals (Thompson, 1987; Champagne, 1988; Selle *et al.,* 2000; Cowieson *et al.,* 2004). Phytic

acid can also form complexes with AAs and digestive enzymes, particularly proteolytic enzymes and will therefore reduce their activity. Due to a shortage of phytase enzyme secretion in the digestive system of birds, phytic acid and its binary and ternary complexes cannot be hydrolysed, therefore, it is considered as an ANF in poultry feed. These binary and ternary complexes limit the bioavailability of nutrients, especially proteins, AAs and minerals for broilers (Selle *et al.,* 2000; Cowieson *et al.,* 2004). These complexes also cause a hyperactive pancreas, due to a physiological negative feedback mechanism, thus increasing the losses of endogenous AAs in the form of digestive enzymes (Selle *et al.,* 2000; Cowieson *et al.,* 2004).

Phytic acid content varies according to legume type (Table 1. 6), cultivar and growing location (Oomah *et al.,* 2011). It has been stated that the level of phytate in field beans can be significantly reduced via breeding (Griffiths and Thomas, 1981). In comparison with that of white-flowered, a wider range of phytate has been reported within colour-flowering field bean cultivars (Table 1. 7) (Makkar *et al.,* 1997; Oomah *et al.,* 2011). The overall phytate phosphorus content average is 53.3% in spring, 69.3% in winter (Duc *et al.,* 1999), 70.9% in white-flowering and 79.2% in colour-flowering (Makkar *et al.,* 1997) field bean cultivars, respectively.

Field beans also contain a phytase enzyme. Oomah *et al.* (2011) found ranges of 1434-2532 and 1171-2151 phytase units/kg DM in white and colour-flowering field bean cultivars, respectively. They also found different concentrations in the same cultivars, grown in different locations.

1. 2. 3. 3. Protease inhibitors

The protease inhibitors that exist among most legume seeds are biochemically classified into two major groups: those that only inactivate trypsin enzyme and those that contain a high rate of cystine that can inactivate both trypsin and chymotrypsin at their free linking sites (Makkar *et al.,* 2007).

Trypsin inhibitors are crystalline globular proteins which inactivate the proteolytic capability of trypsin enzyme. They are located in both cotyledons and testa of field beans (Marquardt *et al.,* 1975; Alonso *et al.,* 2000a) and peas (Wang *et al.,* 2009). The concentration of trypsin inhibitors is different between different legumes (Table 1. 6) and among the cultivars of the same legume type (Table 1. 7).

Soybean meal contains a much higher level of trypsin inhibitors than other beans, followed by peas, then field beans (Table 1. 6) (Jezierny *et al.,* 2011). A range of 0.04-1.99 and 1.11-3.55 g/kg DM of trypsin inhibitors has been detected in colour and white-flowering field bean cultivars, respectively (Makkar *et al.,* 1997; Jezierny *et al.,* 2011). Trypsin inhibitor activity in spring genotypes is more than five-fold higher than that in winter ones (4.7 *versus* 0.8 units/mg DM) (Duc *et al.,* 1999).

Overall, all raw field bean species, such as white and colour-flowering, spring, winter, green hull and white hull field bean cultivars contain lower levels of trypsin inhibitors than those in heat-treated SBM products that are commercially available and widely employed in poultry feeding. Thereby, the impact of trypsin inhibitors in field beans is negligible in broiler diets (Duc *et al.,* 1999).

Chymotrypsin inhibitors are also protease inhibitors found in legume seeds, especially in field beans, which are similar to the trypsin inhibitors in the way they inactivate trypsin and chymotrypsin enzymes and thereby reduce the digestibility of dietary protein. These protease inhibitors lead to a growth depression, due to deterioration of the bioavailability of peptides and AAs for animals (Makkar *et al.,* 2007). Levels of chymotrypsin inhibitors are low and account for 3.56 and 3.71 IU/mg DM in entire field beans and their cotyledons, correspondingly (Alonso *et al.*, 2000a).

1. 2. 3. 4. Amylase inhibitors

Amylase inhibitors, which are concentrated in the cotyledons of legume seeds (Alonso *et al.,* 2000a), are glycoproteins that inactivate α-amylase from different sources, such as saliva, pancreas, bacteria and insects. They restrict the digestibility of starch (Makkar *et al.,* 2007). Makkar *et al.* (1997) did not find any α-amylase inhibitor activity (ICC-units) among twelve different white and colour-flowering field bean cultivars. Also, Abd El-Hady and Habiba (2003) did not detect any amylase inhibitors in field beans and peas, while Alonso *et al.* (2000a) reported 18.9 and 20.7 IU amylase inhibitors per g DM of entire and cotyledons of field beans (correspondingly).

1. 2. 3. 5. Lectins

Lectins are carbohydrate-binding proteins with at least one carbohydrate-binding site that coheres conversely to particular mono or oligosaccharide receptor on cells (Peumans and Van Damme, 1995). All lectins in field beans (Marquardt *et al.,* 1975; Alonso *et al.,* 2000a) and peas (Alonso *et al.,* 1998) are located in their cotyledons. An average of 1.2 g/kg DM lectins in many different field bean genotypes has been reported by Duc *et al.* (1999). Lectin activity in peas, which accounts for 0.006 HU/mg DM (Alonso *et al.,* 2000b), is much lower than 49 HU/mg DM in field beans (Alonso *et al.,* 2000a), and of those levels in soybeans.

The same lectin content in both white and colour-flowering raw field bean cultivars have repeatedly been documented (Table 1. 7). Whereas, Duc *et al.* (1999) noted slightly lower lectin activity in spring than winter field bean cultivars (0.7 *versus* 1.7 g/kg DM).

The high concentration of lectins in raw soybean and their negative effect on growth makes them the main ANF after trypsin inhibitors (Grant, 1988; Grant, 1989; Liener, 1994). Whereas, the haemagglutination activity in raw field beans is very low and does not cause precipitation of chicken erythrocytes (Marquardt *et al.,* 1975; Makkar *et al.,* 1997).

1. 2. 3. 6. Saponins

Saponins are glycosides which have a bitter taste. Francis *et al.* (2002) found negative influences of saponins extracted from pericarps fruit (*Sapindus rarak*) on ruminant animals. Ingestion of saponins causes a reduction of feed intake, thus depressing growth performance of poultry. The saponins of soybeans and field beans are relatively harmless and do not cause hemolysis (Liener, 1994; Makkar *et al.,* 1997).

Generally, field beans contain half the amount of saponins that are found in heat-treated SBM (Makkar *et al.,* 1997). There are trace concentrations of saponins in peas (Allen, 1998), which are much lower than that in field beans and SBM (Table 1. 6). Colour-flowering field bean cultivars contain about 42% more saponins than white-flowering cultivars (Table 1. 7) (Makkar *et al.,* 1997).

1. 2. 3. 7. Vicine and convicin

Vicine and convicine are anti-nutritive compounds that belong to pyrimidine glycosides and are both concentrated in the cotyledons of some field bean cultivars (Champ, 2002; Vilariño *et al.,* 2009; Khamassi *et al.,* 2013).

Legume seeds such as field beans (Duc *et al.,* 1999; Grosjean *et al.,* 2001) and peas (Fru-Nji *et al.,* 2007) contain vicine and convicine compounds. The presence or absence of vicine and convicine in legume seeds is strongly associated with genotype. Duc *et al.* (1989) found a reduction of 10-20-fold in vicine and convicine in field beans following mutation on the *vc*$^{-}$ gene.

The concentration of vicine is higher than convicine in field beans, and white-flowering field bean cultivars contain more vicine, but similar covicine compared to colour-flowering cultivars (Makkar *et al.,* 1997) (Table 1. 7). Moreover, Helsper *et al.* (1993) reported 14% lower levels of vicine and convicine in cotyledons of T+ genotypes compared to those of T- faba bean genotypes. Whereas, Duc *et al.* (1999) detected similar mean of concentrations of vicine and convicine in T+ and T- field bean genotypes (Table 1. 7). These researchers reported means of 9.9 g/kg DM of vicine and 2.4 g/kg DM convicive in winter genotypes, which was dramatically higher than that in spring genotypes that carried *vc*$^{-}$ gene (2.3 g of vicine and 1.4 g convicine/kg DM).

1. 3. Nutrient availability of field beans for broilers

The nutritional quality of field beans for broilers relates to the concentration and digestibility of their nutrient contents, which changes based on the extent of dilution of those nutrient ingredients by compounds of no nutritional value. This is also affected by the interaction with those of negative nutritive impacts such as NSPs, tannins, phytate and vicine and convincine. These compounds (anti-nutrients), which are capable of restricting the utilisation of nutrients and stimulating an increase in endogenous secretion, are extremely nutritionally expensive for poultry, particularly young broilers.

The aim of this part is to review the availability of the main nutrients such as carbohydrates, proteins, amino acids, lipids, minerals and vitamins of field bean cultivars for broiler chickens.

1. 3. 1. Carbohydrate availability

1. 3. 1. 1. Starch availability

Low accessibility of carbohydrase enzymes to starch granules is a frequent characteristic of the majority of starchy legume seeds. It is claimed that the starch granule bodies of field beans are more firmly synthesised compared to those of wheat (Stoisch, 1994). It has also been indicated that in comparison with maize starch granules, those of field beans are more resistant to amylase from pancreatin (Sugimoto, 1980). Starch digestibility of field beans (Totsuka *et al.,* 1977; Lacassagne *et al.,* 1991) and peas (Carré and Brillouet, 1989; Carré *et al.,* 1991) depends on the accessibility of carbohydrase enzymes to the starch granules.

Ileal starch digestibility of 0.795 and 0.815 for faba beans and peas, respectively, has been documented with 35 day old male Ross 308 broilers (Nalle *et al.,* 2010b). Whereas, Métayer *et al.* (2003) stated that the majority of starch in field beans and peas was digested by broilers. They observed similar starch digestibility among peas (97.9%), and tannin-free and vicine and convicine-high (T- VC+) (95.4 and 96.9%), tannin-containing and vicine and convicine-low (T+ VC-) (97.6 and 98.0%) and tannin-free and vicine and convicine-low (T- VC-) (97.5 and 98.5%) field bean cultivars for both 20-24 day old broiler chickens and adult cockerels (1-56 day old birds), respectively when fed in pelleted diets.

Lacassagne *et al.* (1988) noted starch digestibility of 83.4-85.6% in T+ and 75.1% in T- field beans fed at 48% in diets to three week old chicks. Likewise, Lacassagne *et al.* (1991) reported starch digestibility of 70% and 64% for T+ and T- reconstituted field beans (respectively) fed at 41% to 21 day chickens. Whereas, Flores *et al.* (1994) observed that field bean tannins negatively influenced their starch digestibility. Moreover, in three tube-fed experiments conducted by Longstaff *et al.* (1993), it was noticed that faba bean condensed tannins depressed starch digestibility for cockerels. They reported about 8% starch digestibility in T- field beans over that in T+ field beans (0.886 *versus* 0.819 starch digestibility coefficients). The authors found a considerable regression relationship (P<0.001) of bean starch digestibility *versus* condensed tannin rich-testas.

The deleterious effect of tannins on starch digestibility of field beans for broilers relies on the ingested tannin dosage (Longstaff and McNab, 1991a, b; Flores *et al.,* 1994; Grosjean *et al.,* 2000; Vilariño *et al.,* 2009). Lacassagne *et al.* (1988, 1991) suggested that only ingesting high dosages of tannins deteriorate starch digestibility of faba beans for chicks. Longstaff and McNab (1991a, b) found that high concentrations of field bean tannins

decrease the activity of α-amylase from both the jejunum and ileum, thus reducing starch digestibility of field beans for broilers.

1. 3. 1. 2. Non-starch polysaccharide availability

Dietary NSPs are fermented in the gut of broilers by intestinal microflora at a very low extent compared to that of pigs and rats (Jørgensen *et al.,* 1996).

Nalle (2009) documented mean concentrations of about 20 and 195 g/kg DM of S-NSPs and I-NSPs (respectively) in field beans. It has been documented that I-NSPs are indigestible by cockerels, while soluble NSP segments are degradable to an extent depending on bird age, as lower digestibility is seen with younger birds compared to that of adults (Carré *et al.,* 1995b). The S-NSPs are digested in the caeca by the microflora and degraded in the more proximal parts of the small intestine of birds (Carré and Gomez, 1994).

There is a positive relationship between the small intestinal microbial fermentation and the dietary cell wall polysaccharides (Choct *et al.,* 1996), which is detrimental to the performance and well-being of poultry, and the predominant segment of fermentation outcomes such as organic acids are absorbed in the caeca of the birds (Carré and Gomez, 1994).

The average content of cellulose and acid detergent lignin is a respective 115 and 5 g/kg DM in field beans (Jezierny *et al.,* 2011), and it has been stated that cellulose and liginin are completely indigestible in fowls (Bolton, 1955). Xylose content of legume seeds, which is about 16.5 g/kg DM in field beans (Perez-Maldonado *et al.,* 1999) and forms about 8% of field bean NSPs (Rubio *et al.,* 1992), is completely undegradable and unabsorbed by chickens (Carré *et al.,* 1994). Although chickens lack secretion of an enzyme to digest galactose of legume seeds, it is hydrolysed by the gut microflora (Carré *et al.,* 1994) at a high rate in adults (Carré *et al.,* 1995a, b) and to a lower extent in young birds (Carré *et al.,* 1995b).

Griffiths and Jones (1977) in an *in vitro* study noted that tannins highly decreased cellulase solubility of field bean hull, and tannins inactivated cellulase enzyme effect on its substrate.

1. 3. 2. Protein and amino acid availability

Nalle (2009) found slightly higher apparent ileal digestibility of lysine, methionine, alanine and aspartic acid in field beans compared to that of peas, but lower than that in SBM, while digestibility coefficients of the other AA were slightly lower in field beans (Table 1. 8). The digestibility of histidine, cysteine and proline of in field beans were much lower compared to that of the other mentioned legume seeds for five week old broilers (Table 1. 8). Whereas, O'Neill *et al.* (2012) reported significantly lower standardised ileal digestibility

of CP (0.744 and 0.796, respectively) and most AAs in field beans compared to those of peas with four week old broilers.

Generally, the availability of histidine, cysteine, proline, tryptophan and isoleucine is low, but that of arginine, lysine and phenylalanine is high compared to that of the rest of the field bean AAs for broilers (Nalle, 2009; Woyengo and Nyachoti, 2012; O'Neill *et al.,* 2012).

Tannins adversely affect the extent of the utilisation of CP (Tables 1. 9 and 1. 10) (Lacassagne *et al.,* 1991; Vilariño *et al.,* 2009) and AAs (Ortiz *et al.,* 1993; Woyengo and Nyachoti, 2012) in field beans by broilers. A very high negative correlation (P<0.001) has been observed between apparent total tract nitrogen retention (NR) and the tannin content of field beans for young broilers (Vilariño *et al.,* 2009). These researchers have suggested that the adverse effect of tannins on CP availability of field beans for broilers depends on their tannin content levels, with 91% of the difference in protein digestibility of field beans for broilers is due to tannins.

A high negative relationship between dietary condensed tannins and NR (Marqurdt and Ward, 1979) and ileal digestibility of CP and all individual AAs (Ortiz *et al.,* 1993) of field bean for broilers have been documented. Moreover, Ortiz *et al.* (1993) stated that condensed tannins of field beans do not particularly influence a group of AAs or an individual AA. In comparison with T- field beans, T+ cultivars have significantly lower ileal digestibility (Ortiz *et al.,* 1993; Woyengo and Nyachoti, 2012) and retention (Marquardt and Ward, 1979) of all individual AAs for chicks.

A few mechanisms have been suggested for explaining the reduction in the utilisation of protein and AAs of field beans with condensed tannins. For example, formation of tannin-protein complexes that deteriorate protein digestibility by proteolytic enzymes is one mechanism. Proteolytic enzymes may also be inhibited after binding with condensed tannins, thus reducing protein degradation (Longstaff and McNab, 1991a; Mueller-Harvey and McAllan, 1992; Makkar *et al.,* 1993; Makkar *et al.,* 1997). Also, the interaction of tannins with digestion resultants of dietary proteins, such as peptides and AAs is another reason for the deterioration of AA availability of field beans for chicks (Ortiz *et al.,* 1993). Interference between tannins and the gastrointestinal epithelial protective mucus results in the modification of the absorption of digestion products. This may thus be another cause of the decrease in CP and AA utilisation due to tannins (Oh and Hoff, 1986; Butler, 1989).

Field beans contain 4.93-21.38 g/kg DM of phytate (Makkar *et al.,* 1997; Oomah *et al.,* 2011), and inherent binary protein-phytate complexes have been found in legumes seeds (Prattley and Stanley, 1982). These types of complexes may be constituted to a predominant extent in the upper part of the digestive tract of birds as well. Also, phytate probably forms ternary protein-mineral-phytate complexes in the small intestine (Champagne, 1988). Phytate and its binary or ternary complexes are not degradable by birds and negatively affect the utilisation of dietary protein and AAs in various ways. For

example, phytate can conjugate with endogenous and exogenous enzymes, or their substrates (via ternary complex formation). Thereby phytate limits the digestive effectiveness of the enzymes and decrease CP digestibility, utilisation of AAs and stimulate more secretion of digestive enzymes into the small intestines by a negative feedback mechanism, thereby raising the losses of endogenous AAs (Selle *et al.,* 2000; Cowieson *et al.,* 2004).

Furthermore, a negative relationship has been observed between NSPs and *in vitro* protein digestibility in field beans (Duc *et al.,* 1999), which may be due to the limitation of accessibility of proteolytic enzymes to the trapped proteins by cell wall materials. Concentration of NSPs and their proportion to that of CP in field beans is another potential factor that determines the utilisation of their CP and AAs by broilers. The deleterious effect of field bean NSPs on the utilisation of CP and AAs of field beans for young broilers is known (Longstaff and McNab, 1991a; Lacassagne *et al.,* 1991; Nalle *et al.,* 2010b). This may be due to the interaction of NSPs with intestinal cell wall or causing an increase in the secretion of endogenous proteins into the intestines of birds (Padilla *et al.,* 2005).

Table 1. 8. Apparent ileal amino acid digestibility coefficients of field beans, peas and SBM for broilers

	Field beans	Range in field beans	Peas	SBM
Indispensable amino acids				
Arginine	0.893	0.881-0.900	0.914	0.918
Histidine	0.739	0.738-0.739	0.815	0.910
Isoleucine	0.833	0.825-0.842	0.841	0.891
Leucine	0.844	0.831-0.854	0.843	0.876
Lysine	0.891	0.880-0.896	0.888	0.915
Methionine	0.829	0.825-0.834	0.816	0.898
Phenylalanine	0.872	0.851-0.882	0.873	0.888
Threonine	0.790	0.777-0.811	0.781	0.855
Valine	0.815	0.807-0.822	0.835	0.879
Mean of indispensable amino acids	0.834	0.824-0.842	0.845	0.892
Dispensable amino acids				
Alanine	0.877	0.854-0.891	0.837	0.869
Aspartic acid	0.866	0.844-0.885	0.844	0.865
Cysteine	0.564	0.544-0.597	0.648	0.809
Glutamic acid	0.886	0.876-0.891	0.901	0.901
Glycine	0.785	0.782-0.789	0.804	0.853
Proline	0.598	0.569-0.642	0.759	0.887
Serine	0.819	0.808-0.837	0.807	0.869
Tyrosine	0.791	0.774-0.812	0.811	0.895
Mean of dispensable amino acids	0.774	0.772-0.782	0.801	0.869
Average digestibility of 17 amino acids	0.805	0.797-0.814	0.825	0.881

Adapted from Nalle (2009).

1. 3. 3. Availability of fat, vitamins and minerals

Tannin-containing field beans have higher fat availability for broilers than T- cultivars. Marquardt and Ward (1979) found a high positive correlation between condensed tannin content and total tract fat digestibility in field beans for chicks. They reported 75.6 and 79.0% total tract fat digestibility with one week old chicks fed T- and T+ field beans, respectively. They also found an increase in fat digestibility after the addition of pure condensed tannins to chick diets (Table 1. 9). Longstaff *et al.* (1993) noted that fat digestibility of field bean diets was improved for cockerels by condensed tannins when the protein content of the diet was high. It has been ascertained that low dietary concentrations of tannin-containing field bean hulls (20-50 g testa/kg diet) increases the activity of lipase enzyme in the jejunum and ileum of chicks and increases fat digestibility (Longstaff and McNab, 1991a, b).

However, there is no research showing the availability of vitamins from field beans for chickens, but it is generally accepted that the availability of fat soluble vitamins is highly related to the availability of dietary fat, thus any deterioration in the quality and availability of field bean lipids reflects on the utilisation of A, K, E and D vitamins. Since unsaturated fatty acids are the primary constituents in field bean lipids, field bean lipids go rancid rapidly after milling, especially when the milled field beans are stored under room temperature for several days (Blair, 2008), and in consequence the utilisation of lipids, fatty acids and fat soluble vitamins may decrease. In contrast with fat-soluble vitamins, the absorption of water soluble vitamins (possibly excluding thiamine) is not related to that of the lipids.

Table 1. 9. Nutrient retention coefficients in chicks fed diets containing different levels of condensed tannins

	Nutrient retention coefficients				
Pure condensed tannins%	Dry matter	Nitrogen	Fat	Ash	Calcium
0	0.696	0.626	0.843	0.467	0.548
0.7	0.689	0.581	0.867	0.444	0.512
1.4	0.660	0.564	0.852	0.426	0.513
2.8	0.626	0.504	0.885	0.408	0.458
5.6	0.593	0.436	0.918	0.400	0.470

Adapted from Marquardt and Ward (1979).

The concentration of phytate varies from 11.6-20.0 g/kg DM in field beans and about 70.9-79.2 is in a phytate-phosphorus form (Makkar *et al.,* 1997). The formation of ternary protein-mineral-phytate complexes may occur in the small intestine of birds when divalent cations, especially Ca^{2+} are available (Selle *et al.,* 2000), as ternary complexes of phytate, histidine and calcium, zinc and copper at a neutral pH have been observed (Champagne

et al., 1990). There is a general agreement that formation of these complexes restrict partly or completely the availability of minerals for birds. Champagne (1988) mentioned that phytate may limit the utilisation of calcium, zinc and iron via formation of ternary protein-mineral-phytate complexes in the small intestine. It is suggested that field bean cultivars that contain higher phytate phosphorus such as winter (Duc *et al.,* 1999) and colour-flowering cultivars also have lower phosphorus availability for animals (Makkar *et al.,* 1997).

In the testa of field beans, the majority of zinc and low rate of iron is chelated in phytate-fibre complexes. Whereas, in cotyledons of field beans, relatively all the zinc is chelated by phytate, while iron is chelated by both phytate and cell fibres (Luo *et al.,* 2010). An *in vitro* study has shown that field bean phytate decreases its iron bioavailability (Luo *et al.,* 2012). The availability of phosphorus from legume seeds is low and variable, depending on the legume type and is approximately 25 and 35% in field beans and peas, respectively (Sauveur, 1989).

In the testa of field beans, most of the iron is chelated into an iron-tannin complex (Luo *et al.,* 2010). A high negative correlation has been found between the concentration of condensed tannins and retention of ash and calcium by young chicks (Marquardt and Ward, 1979). They noted 33.7 and 24.3% ash retention with chicks consuming T- and T+ based field bean diets, respectively. They also found a decrease in calcium and ash retention following the addition of pure condensed tannins in chick diets (Table 1. 9).

1. 3. 4. Apparent metabolisable energy

Makkar *et al.* (1997) in an *in vitro* degradability test found significantly (P<0.001) higher metabolisable energy (ME) values in white-flowering than those with colour-flowering field bean cultivars and SBM, with mean averages of 14.0, 12.3 and 13.1 MJ/kg DM, respectively. It is generally accepted that tannins, vicine and convicine depress the ME of faba beans fed in mash or pelleted form to broilers (Table 1. 10).

Brufau *et al.* (1998) documented that T- field bean cultivars contained 0.73 MJ/kg DM higher nitrogen-corrected apparent metabolisable energy (AMEn) than T+ field beans (11.49 *versus* 10.76 MJ/kg DM). Lacassagne *et al.* (1988) reported 0.3 MJ/kg DM of AMEn in T- above that in T+ faba beans when fed in mash or pelleted form diets to broilers (Table 1. 10). It has been documented that the AMEn value of T- VC- is 0.77, 0.56 and 0.53 MJ/kg DM higher than that found in tannin-containing and vicine and convicine-high (T+ VC+), T+ VC- and T- VC+ reconstituted faba beans, respectively, when fed as mash to broilers (Table 1. 10) (Vilariño *et al.,* 2009). Similarly, Métayer *et al.* (2003) observed values of 0.76 and 0.38 MJ/kg DM higher AMEn in T- VC- than in T- VC+ and T+ VC- faba beans, respectively, when fed in pelleted diets to broilers (Table 1. 10).

Table 1. 10. Nitrogen-corrected apparent metabolisable energy (AMEn) and apparent protein digestibility coefficient (APD) contents of field beans different in tannin and vicine and convicine contents fed as mash and pelleted to broiler chickens

Field bean cultivar	Fed as mash	Fed as mash	Reference	Fed as pelleted	Fed as pelleted	Reference
	AMEn	APD		AMEn	APD	
T- VC-	12.74	0.879	3	12.70	0.893	2
T- VC+	10.17	0.826	1	11.94	0.906	2
	12.21	0.850	3	11.80	0.872	1
T+ VC-	12.18	0.768	3			
				12.32	0.826	2
T+ VC+	10.47	0.682	1	11.50	0.715	1
	11.97	0.741	3			

Adapted from [1]Lacassagne *et al.* (1988); [2]Métayer *et al.* (2003); [3]Vilariño *et al.* (2009); T- VC-, tannin-free and vicine and convicine-low; T- VC+, tannin-free and vicine and convicine-high; T+ VC-, tannin-containing and vicine and convicine-low; T+ VC+, tannin-containing and vicine and convicine-high field bean cultivars.

1. 4. Strategies for improving the feeding value of field beans for broilers

Field beans are a reasonable source of proteins, AAs and energy, but their exploitation in broiler diet formulations is still not efficient. This may be due to a lack of knowledge of how to reduce the variability in their energy and nutrient availability for broilers. Field beans may contain different levels of compounds with anti-nutritional effects, which reduce their nutritional quality for broilers (Vilariño *et al.,* 2009). There are several strategies that can decrease or remove the anti-nutrient contents of field beans, thus improving their nutritional value for broilers. Plant breeding, exogenous enzyme supplementation, physical processing such as mechanical processing and thermal treatments are methods that may ameliorate the nutritional value of field beans for poultry. The improvement in the nutritional value of field beans for broilers is related to increasing their energy, protein and AA bioavailability.

1. 4. 1. Plant breeding

The feeding value of legume seeds relates to their composition in terms of nutrients, anti-nutrients, materials of no nutritional value and their proportion to each other. Duc *et al.* (1999) and Oomah *et al.* (2011) found a possibility of increasing the concentration of nutrients and decreasing the content of anti-nutrients in field beans via breeding (genotype, as well as location of cultivation determined the chemical composition of the field beans). Subsequently, the produced cultivars from the study conducted by Duc *et al.*

(1999) were used in a feeding study by Grosjean *et al.* (2001), who noticed that breeding significantly increased energy and nutrient availability of the field beans for simple stomach animals.

Tannin-free field bean cultivars, particularly spring-grown ones, contain considerably higher amounts of all nutrients and lower levels of most ANFs than T+ cultivars (Makkar *et al.,* 1997; Duc *et al.,* 1999). Tannin-free field bean cultivars result in high protein, AA (Woyengo and Nyachoti, 2012; O'Neill *et al.,* 2012), dry matter and mineral availability (Marquardt and Ward, 1979), and also higher AMEn (Metayer *et al.,* 2003; Vilariño *et al.,* 2009) for broilers compared to T+ cultivars. Also, feeding diets based on T- and low vicine and convicine field bean cultivars lead to higher AMEn for broilers compared to those T+, vicine and convicine compounds alone or together (Metayer *et al.,* 2003; Vilariño *et al.,* 2009). Thereby, there is the possibility of enhancing the chemical composition, thus the nutritional quality of field beans for broilers via breeding and employing zero tannin genes (*zt*2) and the low vicin-convicine gene (vc^-), which are now available on the market.

1. 4. 2. Exogenous enzyme inclusion

Exogenous enzyme involvement in poultry diets is considered as an effective strategy for reducing the detrimental impacts of dietary anti-nutrients, thus enhancing the nutritional quality of diets. Experimental results have shown that dietary energy and nutrient utilisation of legume seeds is improved by enzyme addition. The improvement in the nutritional value of grain legumes for broilers, due to enzyme impacts, is connected with increasing their energy and protein availability (Wiryawan and Dingle, 1999). The beneficial effects of enzymes on enhancing the nutritive value of feedstuffs depend on many factors, such as the type, source and dosage of the enzymes, the concentration of their substrates, the inherent availability of the nutrients in the feedstuff and the manner under which the feed ingredient is in the diet (Castanon and Marquardt, 1989; Cowieson *et al.,* 2006). High doses of enzymes, such as xylanase have deleterious effects due to their interaction with endogenous enzymes and denaturation of intestinal cell wall proteins (Sahraei and Ghazi, 2012). Castanon and Marquardt (1989), however, noticed that supplementation of suitable doses of protease and cellulase individually and together increased the nutritional value of raw field beans for chicks but did not cause any improvement when they were added to diets containing fermented or autoclaved field beans. A lack of significant improvement in the nutritive value of diets based on fermented or autoclaved field beans with the enzyme addition may be due to the existing of limited amount of substrates, particularly NSP, in the fermented and autoclaved beans.

Sahraei and Ghazi (2012) found that supplying different doses of xylanase to dehulled faba bean based diets did not alter AME or true metabolisable energy (TME), but did improve their protein digestibility, depending on the enzyme dosage. They also noticed a negative effect of super doses of the enzyme on protein digestibility of field bean based

diets. The authors suggested that the improvement in protein utilisation may have resulted from hydrolysing the cell wall materials and releasing previously encapsulated protein. The lack of improvement in AME, however, was probably due to the low levels of substrate in the cotyledons after removing the highly fibrous seed-coat, which is known to significantly decrease the ME content of field beans for broilers. Enhancements of 22% in AME and 9.6% in dry matter digestibility were documented in faba bean containing diets following the inclusion of an enzyme that had multi-carbohydrase activities, including pectinase and hemi-cellulase (Hughes *et al.,* 2002).

Wiryawan *et al.* (1995) observed a significant improvement in the TME of a growing chick diet contained field beans, following inclusion of xylanase at the rate of 1 g/kg of diet. Wiryawan *et al.* (1997) also observed significant (P<0.01) amelioration in the net protein ratio (NPR) for faba beans following inclusion of 1 g/kg diet of a multi enzyme product containing xylanase, α-amylase and protease. The authors also noted a considerable (P<0.05) positive correlation between the NSP content of legume seeds and improvement of NPR means due to the enzyme product supplementation. Moreover, Cowieson *et al.* (2003) observed a significant increase in the nutritional value of diets containing 30% pea meal (from many different cultivars) for broilers following the supply of a cocktail of carbohydrases, biosynthesised from *Aspergillus niger* (1000, 2000 and 3000 U/kg of cellulase, α-amylase and xylanase, correspondingly).

In an *in vitro* study conducted by Luo *et al.* (2012) it was found that phytase improves iron bioavailability of field beans. The results have shown that up to 93% of zinc in the kernels of field beans can be available by involving phytase. Also the availability of zinc in field bean hull and iron in both testa and cotyledons can be enhanced with a mixture of cellulase and phytase (Luo *et al.,* 2010).

Although the impact of exogenous phytase on the nutritional value of different feedstuff has been studied, available information on its effect on field bean based diets for broilers is lacking. However, tannins are considered as the main anti-nutrient in field beans that reduce their energy and protein availability for broilers (Vilariño *et al.,* 2009). Presently, there is no available published research illustrating the influence of an enzyme product that hydrolyses tannins on the feeding quality of field beans for broilers.

1. 4. 3. Physical processing

1. 4. 3. 1. Mechanical treatments

Grinding and dehulling are two mechanical strategies that enhance the feeding value of field beans for broilers.

1. 4. 3. 1. 1. Degree of grinding

It has been stated that the low digestibility of field bean starch may be associated with its granulose structure (McEwen *et al.,* 1974). The extent of starch digestibility for field beans (Totsuka *et al.,* 1977; Lacassagne *et al.,* 1991) relies on the degree of grinding and the surface area of the field bean meal particles, which determines the accessibility of carbohydrase enzymes to the starch granules. Fine grinding increases the surface area of field bean meal particles and facilitates the accessibility of digestive enzymes to their substrates (Lacassagne *et al.,* 1991), thus improving the utilisation of their nutrients, particularly starch.

Generally, there is limited published research showing the impact of different degrees of grinding on the nutritional value of field beans for broilers. Lacassagne *et al.* (1991) ascertained that the degree of grinding did not cause a potential change in apparent protein digestibility of field beans, but grinding to 0.16 mm improved starch digestibility and AMEn by more than a quarter over those of 0.5 mm particle size. These authors documented increases of 28.3 and 26.0%, and 22.2 and 32.3% in starch digestibility of T+ and T- reconstituted faba beans and their cotyledons, respectively, following the exchange of a 0.50 mm with a 0.16 mm meal mesh with three week old chicks fed 41% field beans in their diets. Fine grinding (0.16 mm) also resulted in increased AMEn by 2.83 and 2.03 MJ/kg DM for T+ and T- in reconstituted faba beans (respectively), and by 3.55 and 2.97 MJ/kg DM in cotyledons of T+ and T- faba beans, respectively over those 0.50 mm ground ones (Table 1. 11) (Lacassagne *et al.,* 1991). Compared to normal grinding (0.50 mm) reconstituted whole seeds, fine grinding (0.16 mm) dehulled field beans resulted in increases of 5.39 and 4.29 MJ/kg DM in AMEn for T+ and T- field bean cultivars, respectively (Table 1. 11) (Lacassagne *et al.,* 1991).

Table 1. 11. Effect of tannins, dehulling and degree of grinding on apparent digestibility of proteins (ADP) and starch, and apparent metabolisable energy in faba beans for three week chicks

		Reconstituted whole seeds		Dehulled	
Variable	Degree of grinding	T+[1]	T-[2]	T+	T-
ADP	Normal	0.694	0.842	0.820	0.811
	Fine	0.698	0.825	0.850	0.799
Starch digestibility	Normal	0.703	0.638	0.744	0.607
	Fine	0.902	0.804	0.909	0.803
AMEn (MJ/kg DM)	Normal	9.55	9.20	11.39	10.52
	Fine	12.38	11.23	14.94	13.49

Adapted from Lacassagne *et al.* (1991); [1]T+, tannin-containing; [2]T-, tannin-free.

1. 4. 3. 1. 2. Dehulling

Fibres form more than half of the field bean seed-coat (Marquardt *et al.,* 1975). It has been found that about 53, 55 and 30% of T-NSPs, I-NSPs and S-NSPs (respectively) are concentrated in the hull of field beans (Nalle *et al.,* 2010b). Longstaff and McNab (1991a) and Rubio *et al.* (1992) found that glucose (as cellulose and hemicellulose) forms about half of the faba bean hull NSP constituent sugars (Table 1. 12). Thereby, the primary purpose of dehulling is reducing the indigestible highly fibrous part and therefore increasing the concentration of starch, CP and AA in field beans (Rubio *et al.,* 1992; Nalle *et al.,* 2010b). An increase in CP (by 15.8%) (Alonso *et al.,* 2000a), starch (by 13.7%) and AA content in field beans (Nalle *et al.,* 2010b) has been obtained with dehulling.

Removing the majority of phenols and condensed tannins is the aim of dehulling colour-flowering field bean cultivars, in addition to decreasing their NSP content (Griffiths and Jones, 1977; Longstaff *et al.,* 1993). Dehulling removes 92.3 and 81.6% of condensed tannis and phenolic compounds (respectively) in field beans (van der Poel *et al.,* 1991; Alonso *et al.,* 2000a).

Table 1. 12. Carbohydrate composition (g/kg DM) of field beans

Traits	Whole seed[2]	Cotyledon[2]	ICR[2]	Hulls[2]	Hulls[1]
Constituent sugar					
Glucose	83	27	40	450.2	567.1[a]
Uronic acids	35	30	36.5	112.4	149.7
Xylose	13.4	5.5	8.6	71	103.7
Galactose	10.8	18	24.4	22.9	19.4
Arabinose	19.6	23	33.2	16.9	13.1
Rhamnose	2.2	1	-	6.2	6.1
Mannose	1.2	0.7	-	2.6	2.1
Fucose	1.6	1	-	3.4	2.0
T-NSPs	166	106	143	686	863.2
Starch	421	486	683	37	-
Free sugars	42	51	I.0	12	-

Adapted from [1]Longstaff and McNab (1991a); [2]Rubio *et al.* (1992); T-NSPs, total non-starch polysaccharides; ICR, insoluble cotyledon residue; -, not determined; [a]cellulose+hemicellulose.

An *in vitro* study showed that organic matter and neutral detergent fibre digestibility of field beans can be increased to 99.27 and 90.33%, respectively, by dehulling (Ferruzzi *et al.,* 2009). It has been documented that the hull of field beans reduces the availability of dietary CP, AAs and starch by the inhibition of digestive enzymes via formation of tannin-enzyme complexes in the gut of chicks or adsorption of the enzymes, depending on their tannin content (Longstaff and McNab, 1991a, b).

Lacassagne *et al.* (1991) found that dehulling improved only protein and starch digestibility of T+, but enhanced AMEn of both T+ and T- field beans when fed at 41% in feed to three week old chicks (Table 1. 11). Similarly, Longstaff *et al.* (1993) found higher starch digestibility and metabolisable energy with tannin-free than those of tannin-containing field beans. They reported the same starch digestibility and ME with cockerels fed only kernels derived from either condensed tannin-containing or condensed tannin-free field bean cultivars, which were higher than those found with their entire beans. Furthermore, Nalle *et al.* (2010b) reported a significant increase in ileal starch digestibility (by 12.6%), AMEn (by 1.6 MJ/kg DM), and ileal digestibility of histidine, cysteine and proline of faba beans due to dehulling. They also achieved numerical increases in ileal digestibility of other AAs for five week old broilers due to dehulling. It was suggested that the increase in AMEn and AA utilisation of dehulled faba beans is related to removing the indigestible and highly fibrous seed-coat, thereby increasing the concentration of nutrients, such as starch, protein and AAs. However dehulling is an effective method for removing condensed tannins and reducing phenols and NSPs, but it does not decrease other ANFs in field beans (Alonso *et al.,* 2000a).

1. 4. 3. 2. Thermal processing

The temperature, duration of heating and the level of heat-labile anti-nutrients in feedstuff determine the effectiveness of the heating process and its consequences. It is therefore crucial to exploit a suitable temperature and duration of treating legume seeds, which optimises the enhancement of the nutritional quality for broilers (van der Poel, 1990; Araba and Dale, 1990; Kratzer *et al.,* 1990).

Overheating causes protein aggregation, which results in a limitation of protein digestibility (Deshpande and Damodaran, 1989; Dänicke *et al.,* 1998; Carbonaro *et al.,* 2005). Moreover, overheating probably denaturate AAs or modifies the nature of their structure to form indigestible complexes, thereby reducing their availability (Mutia *et al.,* 1994; Wiryawan and Dingle, 1999; Moughan, 2003). Various heat treatments, such as dry and wet heating methods can be exploited for decreasing the concentration of anti-nutrients and improving energy and nutrient utilisation of field beans for broilers.

1. 4. 3. 2. 1. Dry thermal processing

1. 4. 3. 2. 1. 1. Micronising

Micronising is a heating process in which feedstuffs is exposed to a certain wavelength (short, medium or long) of radiation for a certain time. The deployed time and wavelength determine the changes that may happen in the treated feedstuff. This heating treatment causes denaturation of field bean proteinaceous heat-labile anti-nutrients. For example,

heating at 110 ^{o}C for 40 minutes can destroy complete trypsin inhibitors in field beans (Wilson *et al.,* 1972).

Compared to raw field beans, a reduction of 91.57% in trypsin inhibitors and an increase of 23.65% in carbohydrate availability of field beans has been obtained for young chicks fed diets containing 30% micronised field beans (McNab and Wilson, 1974). They also noted significant increases in nitrogen retention and apparent dry matter digestibility due to this treatment. Also, increases of 1.08 and 0.55 MJ/kg in AMEn of individual field beans and whole diets containing 50% field beans have been achieved with micronising (McNab and Wilson, 1974). These researchers also noticed considerable enhancement in feed efficiency due to this heating process. They claimed that an enhancement in feed efficiency, energy and nutrient utilisation of field beans for young chicks following micronising may partially be due to destroying their trypsin inhibitor activity and raising the carbohydrate availability. Moreover, Laudadio *et al.* (2011) found that micronising improved the nutritional value of field beans and they noticed that replacing SBM with 31% micronised (130^{o}C for 1.5 minutes) dehulled-field beans resulted in the same broiler chick performance.

Furthermore, Igbasan and Guenter (1996) reported significant improvements in AME, apparent protein and starch digestibility of peas for broiler chickens as a consequence of using infrared radiation treatment.

1. 4. 3. 2. 2. Wet thermal processing

Pelleting, autoclaving and extrusion are the most commonly used wet thermal processing methods for improving the nutritional value of legume seeds for poultry.

1. 4. 3. 2. 2. 1. Pelleting

Pelleting is an effective way for improving the energy and nutrient availability of field bean-containing diets. Pelleting increases the starch and protein digestibility of faba beans for young chickens (Lacassagne *et al.,* 1991). Lacassagne *et al.* (1988) found improvements of 1.03 and 1.63 MJ/kg DM, 10.65 and 19.44% and 4.84 and 5.57%, in AMEn and apparent digestibility of starch and protein for diets containing 48% of T+ and T- faba bean cultivars, respectively, with broilers by steam pelleting (66-71^{o}C before and 86-87^{o}C after the dies). Also, Grosjean *et al.* (2000) reported significantly higher AMEn, and apparent digestibility of starch and protein of T+VC+, T-VC+ and T-VC- field bean cultivars for adult cockerels, fed in a pelleted state than those of the same cultivars fed in mash form. It is suggested that improvements in AME, the digestibility of starch and protein following pelleting may be related to changing the crystalline structure of starch granules and the enhancement in breaking down the cell walls of the kernels in legume seeds, which improves the accessibility of endogenous enzymes to their substrates (Carre *et al.,* 1991), in addition to reducing their heat-labile ANF contents.

1. 4. 3. 2. 2. 2. Autoclaving

Autoclaving destroys trypsin inhibitor activity and lectins in field beans (Fasina *et al.,* 2003; Luo and Xie, 2013) and may also reduce tannins in faba beans (Luo and Xie, 2013). Ward *et al.* (1977) remarked that there are increases in total tract nitrogen, fibre and dry matter retention, and the performance of young chicks fed autoclaved faba bean hull or entire faba beans over those fed in their raw states. However, Guillaume (1978) documented an increase in starch digestibility of T+ field beans for broilers, but he did not report any considerable change in that of T- field bean based-diets due to autoclaving. Whereas, Brufau *et al.* (1998) and Marquardt and Ward (1979) found that compared with raw, feeding autoclaved T+ or T- field beans caused better nutrient utilisation for broilers. Brufau *et al.* (1998) reported increases of 9.1, 15.6 and 10.9% of T+ and 4.7, 5.8 and 5.2% of T- field beans in the average of essential, non-essential and total AAs, respectively, for chicks, as consequences of autoclaving. Similarly, Marquardt and Ward (1979) found that autoclaving led to significant increases in total tract dry matter (by 32.9 and 22.5%), nitrogen (by 33.2 and 14.4%), total AA (by 16.7 and 8.4%), ash (by 55.6 and 11.9%) retention and fat (by 5.3 and 9.8%) digestibility of T+ and T- faba beans for chicks, respectively, over their raw states.

Shannon and Clandinin (1977) reported an improvement of 15% in ME when whole field beans fed at 250 g/kg of diet due to autoclaving at 121^{o}C for 45 and 60 minutes. They concluded that autoclaving field beans at 121^{o}C resulted in a marginal amelioration in their feeding quality for broilers, but it may not be economical or practical. Castanon and Marquardt (1989) found that compared to the raw state, feeding autoclaved field beans at 907 g/kg to 7-21 days old chickens led to enhancements of 13.9 and 16.3% in dayly weight gain and feed conversion efficiency, respectively.

It has been suggested that improvements in the nutritional value of field beans following autoclaving come from demolishing condensed tannins, other heat-labiled ANFs and modifying the structures of NSPs and starch granules in field beans (Marquardt *et al.,* 1974; Marquardt and Ward, 1979; Brufau *et al.,* 1998).

1. 4. 3. 2. 2. 3. Extrusion

Extrusion is a process in which the diet undergoes mixing, shearing, heating under high pressure and pushing the extrudite through the dies of an extruder (Sørensen *et al.,* 2002). The primary purpose of extrusion is to obtain a greater extent of gelatinised starch and division of seed structure. Also, the denaturation of proteinaceous ANFs is another additional objective (Kearns *et al.,* 1994; Sheriff and Sajeev, 2005; Adamidou *et al.,* 2011). Factors such as the sort of extruded feedstuff, extruding conditions (Diaz *et al.,* 2006; Adamidou *et al.,* 2011), degree of grinding, type of extruder and the sort of employed reactants determine the extent of alteration in extruded dietary ingredients (Björck and Asp, 1983; llo *et al.,* 1996; Grela *et al.,* 2001; Anguita *et al.,* 2006).

Extrusion is an effective method to eliminate trypsin inhibitors, total phenols, tannins, condensed tannins (Masoero *et al.,* 2005; Adamidou *et al.,* 2011) and phytic acid (Abd El-Hady and Habiba, 2003; Adamidou *et al.,* 2011) in field beans. Adamidou *et al.* (2011) found that a reduction in tannin contents in faba beans with extrusion depended on both employed temperature and duration of processing. Alonso *et al.* (2000a) found an increase of 23.5% in *in vitro* protein digestibility of faba beans by extrusion. Over extruding may cause protein aggregation (Carbonaro *et al.,* 2005), destruction of AA or Maillard reactions (Björck and Asp, 1983; Vasanthan *et al.,* 2002), thus a deterioration of protein and AA digestibility may result (Alonso *et al.,* 2000b; Nalle, 2009).

Adamidou *et al.* (2011) found that extrusion resulted in a slight decrease in total, soluble and insoluble NSP contents of field beans. It is claimed that the redistribution of S-NSP segments is probably associated with fragmentary solubilisation or depolimerisation of hemicellulose and insoluble pectic materials (Vidal-Valderde *et al.,* 1992). Alonso *et al.* (2000a) reported an improvement of 82.4% in *in vitro* starch digestibility of faba beans by extrusion. Pérez-Navarrete *et al.* (2006) stated that the enhancement of starch digestibility following extrusion may be related to modifying the structure of starch, for example, fusion, gelatinisation, fragmentation and dextrinisation. Alonso *et al.* (2000a) stated that extrusion changes the starch of legume seeds to a gelatinised pattern by ruining and melting the crystalline structure of starch, due to high temperature and pressure. Thereby, starch digestibility is improved via increasing accessibility of α-amylase to starch compounds.

Kushwah *et al.* (2002) found that extrusion of field beans improved their protein utilisation and growth of rats, particularly when fed at 20% in diets. Whereas, Nalle (2009) found that extrusion cooking increased both *in vitro* and apparent ileal starch digestibility, but did not cause any alteration for AME, AMEn and apparent ileal protein digestibility of peas fed to 4-5 week old male Ross 308 broilers. The amelioration of nutrient availability following extrusion may be associated with destruction of anti-nutrients, changing the structure of dietary NSPs and starch (gelatinisation of starch) (Alonso *et al.,* 2000a; Abd El-Hady and Habiba, 2003; Sheriff and Sajeev, 2005; Adamidou *et al.,* 2011).

1. 5. Conclusion

Exploitation of field beans in broiler diets is not new. Nowadays, field beans are produced in high amounts in the UK. Relatively high differences in the chemical composition of various field bean cultivars have been documented. Starch forms the predominant amount of carbohydrate in field beans. Compared to SBM (as a commonly used source of protein in poultry diets), field beans contain less CP, but a much higher content of starch, while their NSP concentration is similar. Generally, field beans are rich in CP and their AA profile is close to that of SBM.

High differences in the energy and nutrient availability among field bean cultivars for broilers have been reported. The chemical composition and nutritional quality of field beans for broilers differ depending on field bean cultivar and environment under which they are grown. Although there is a high concentration of starch and protein in field beans, their availability for broilers is low and significantly variable depending on their chemical composition. It has been proved that anti-nutrients, especially tannins and NSPs, in field beans reduce their nutrient utilisation for broilers.

The impact of different processing methods on the nutritional value of field beans for broilers has been examined. The methods are different in terms of their advantages and applicability. It has been found that processing techniques, such as fine grinding, dehulling, micronising, autoclaving, pelleting and extrusion, may have some beneficial influences on the nutritive value of field beans for broilers.

Since recent field bean cultivars contain low levels of heat-labile anti-nutrients, such as trypsin inhibitors and lectins and their adverse impact is low on poultry, the beneficial influence of dry heat processing, such as micronising, roasting and microwaving, may be limited for improving their nutritive value for broilers. Although wet heating processes, such as autoclaving and extrusion may improve nutritive value of field beans for broilers, they are complex methods and not very practical in poultry feed formulation. In addition to the fact that dehulling does not lead to removing all ANFs in field beans and does not optimise their energy and nutrient availability, it is an additional process which increases the cost of diet formulation. Thereby, these methods are not considered as very desirable ways for increasing the nutritional value of field beans for poultry.

It has been ascertained that the inclusion of suitable doses of enzymes (which is more practical in diet formulation) that are commonly available and widely used in poultry feed formulation may improve the nutritive value of field beans for broilers. Since NSPs, phytate and tannins decrease dietary energy and nutrient availability for broiler chickens, the inclusion of commercially available enzymes may increase the nutritional value and reduce the variability of energy and nutrient availability of field bean-based diets for broilers.

Nowadays, there are many new field bean cultivars available in the UK market, but their chemical composition, physical characteristics and nutritive values for broilers has not been investigated. There is also a shortage of studies to determine the relationship between the energy and nutrient availability of different field bean cultivars for broilers and their chemical composition and physical properties.

Research conducted on exogenous enzyme supplementation, particularly phytase and tannase, in field bean-containing diets for broilers is limited. Data on the influence of exogenous carbohydrase and protease on the nutritional quality of field beans and their interaction on field bean cultivars is also considerably limited. Additionally, there is no information on the interactions between enzyme supplementation and heat-prosessing

(for example micronising) on the nutrient availability of field beans for broilers. Therefore, the aim of this project is to fill the knowledge gap by characterising the chemical composition and physical properties of new field bean cultivars that are cultivated in the UK and determine if differences exist among cultivars. The inherent energy and nutrient availability of ten UK field bean cultivars for broilers, as well as relationships between the compositional profiles of the experimental field beans and their inherent energy and nutrient availability, will be investigated. Also, the influence of various exogenous enzymes and/or micronising on the nutritional quality of field beans for broilers will be studied.

CHAPTER 2: CHEMICAL QUALITY AND PHYSICAL CHARACTERISATION OF TEN UK GROWN FIELD BEAN (*Vicia faba* L. var. *minor*) CULTIVAR SAMPLES

2. 1. Introduction

Due to their high protein content, field beans are considered an alternative protein source for farm animals, but their use in poultry diets is limited. A wide range of variation in the chemical composition, especially anti-nutrients, is one of the main problems associated with feeding field beans to poultry (Makkar *et al.,* 1997; Crépon *et al.,* 2010).

There are many field bean cultivars produced in the UK. However, information is only available about their agronomic (growing season, flower colour, hilum colour, earliness of ripening, straw height and standing ability at harvest) and seed characteristics (yield and seed weight). Therefore, the main objectives of this study were to determine the overall mean and the variability in the chemical composition (including nutrient and anti-nutrient concentrations, and energy content) and physical characteristics of ten UK grown field bean cultivars. Additional aims were to investigate the relationship between the measured physical variables and relate these variables to the nutritional value of these field bean cultivars for growing broiler chickens, in a latter feeding experiment. These ten field bean cultivars were selected due to their dominance in the UK market.

2. 2. Materials and methods

2. 2. 1. Field bean samples

Ten different UK grown field bean cultivar samples (Figure 2. 1), including three spring (Fuego, Fury and Maris Bead) and seven winter grown (Arthur, Buzz, Clipper, Divine, Honey, Sultan and Wizard), from 2013 harvest year were characterised in this study.

Figure 2. 1. The studied field bean cultivars

2. 2. 2. Laboratory analysis of the field bean samples

The main chemical constituents, including nutrient and anti-nutrient components, and physical properties of the field bean samples were determined. Freshly milled field bean seed samples (200 g) in a hammer-mill passing a 0.8 mm pore diameter sieve were used for analysis. Due to the high levels of nutrients in the field bean samples, all nutrient analyses were performed in duplicate, while analyses were performed in triplicate for anti-nutrient components (due to their low levels and nutritionally negative impacts in broiler diets). All determined variables were expressed on a dry matter basis. The analyses were conducted at Harper Adams University laboratories or as otherwise mentioned.

2. 2. 2. 1. Proximate analysis and gross energy

Dry matter (DM) was determined by drying samples at 105°C to a constant weight in an oven (Genlab, UK Ltd) (AOAC, 1990; 925.10). Ash was measured by ashing samples in a muffle furnace at 500°C for 18 hours. Crude protein (N x 6.25) concentration in the samples was determined by the dry combustion method, using a Leco (FP-528 N, Leco Corp., St. Joseph, MI). Oil (as ether extract) was extracted with diethyl ether by the extraction method (AOAC, 2000), employing a Soxtec system (Foss UK Ltd). Gross energy (GE) was measured using a Parr adiabatic bomb calorimeter (Parr-6200 Calorimeter, Parr Instruments Company, Moline, IL, USA), and benzoic acid was used as the standard.

2. 2. 2. 2. Amino acids

The content of amino acids in the field bean samples was determined following the EC directives 2000/45/EC for tryptophan (OJEU, 2000), and EC/98/64 (L 257/16) for the rest of the amino acids (OJEU, 1998), by using SSNIFF Spezialdiaten GmbH (Soest, Germany). The amino acid analysis was performed by Wonder Media Ltd, DM Scientific-Main Site, Dalton, Thirsk, North Yorkshire, YO7 3JA, UK. The determined values were expressed as g/kg DM and g/kg CP.

2. 2. 2. 3. Carbohydrate analysis

Carbohydrate composition of the studied field bean cultivars was determined by Englyst Carbohydrates, 2 Venture Road, Southampton Science Park, Southampton, SO16 7NP, UK.

2. 2. 2. 3. 1. Total starch

Total starch content of the field bean samples was determined by applying the method suggested by Englyst *et al.* (2000), which involved initial heat dispersion together with heat stable amylase followed by treatment with alkali to disperse any retrograded type III resistant starch. A pH 4.5 buffered aliquot was treated with amyloglucosidase to release

glucose which was quantified by High Performance Liquid Chromatography (HPLC) with pulsed amporimetric detection.

2. 2. 2. 3. 2. Non-starch polysaccharides

Non-starch polysaccharide content of field beans was determined by the method of Englyst *et al.* (1994), where total starch was completely dispersed and then hydrolysed enzymatically. The NSPs were isolated by precipitation in 80% ethanol then hydrolysed by sulphuric acid and the released constituent sugars were estimated by gas chromatography as their alditol acetate derivatives.

2. 2. 2. 4. Minerals

The mineral contents of the field bean samples were determined according to the procedure described by Tanner *et al.* (2002), employing inductively coupled plasma emission spectrometry (Optima 4300 DV Dual View ICP-OE spectrometer, Perkin Elmer, Beaconsfield, UK). The samples were analysed by Analytical Services Department, SAC Consulting, Bush Estate, Penicuik, Midlothian, Scotland, EH26 0QE. UK.

2. 2. 2. 5. Phytate

The phytate content in the field bean samples was determined by HPLC using the procedure described by Kwanyuen and Burton (2005) at Danisco (UK) Ltd, PO Box 777, Marlborough, Wiltshire, SN8 1XN, UK.

2. 2. 2. 6. Trypsin inhibitors

Trypsin inhibitor content in the field beans was measured by applying the assay suggested by Smith *et al.* (1980), as presented in appendix 1.

2. 2. 2. 7. Phenols

Phenolic compounds, including total phenols, non-tannin phenols and total tannins (all as tannic acid equivalents), in the representative samples of the field beans were measured chemically by following the suggested method by Makkar *et al.* (1993) spectrophotometrically, after extraction of phenolic compounds from samples as following. Exactly 400 mg of freshly ground bean seeds (passed through 0.8 mm mesh and freeze-dried) were weighed into a 50 ml plastic tube. 70% aqueous acetone was added to bring the volume to 20 ml; the supernatant was then collected into clean tubes following centrifugation (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) for 10 minutes at 3000 x*g* at 4^{o}C. The tube containing the tannin extract was kept on dry ice until all phenolic analyses were completed during the same day.

2. 2. 2. 7. 1. Total phenols

Total phenols were determined as follows: 50 µl of tannin extract (prepared in section 2.2.2.7) was pipetted into a 10 ml plastic test tube, 950 µl of distilled water, 0.5 ml of 1N Folin-Ciocalteu reagent (freshly prepared from commercially available Folin-Ciocalteu reagent (2N) was diluted with an equal volume of distilled water) and 2.5 ml of 20% sodium carbonate solution (50 g of Na_2CO_3 $10H_2O$ dissolved in 250 ml volumetric flask with distilled water) were then added, respectively. After vortexing and then keeping the tube for 40 minutes at room temperature under a dark condition, the upper part was gently pipetted with a disposable plastic pipette into a cuvette and optical density at 725 nm was read in a spectrophotometer against a blank (Beckman DU 650 spectrophotometer, Beckman instruments, INC. Fullerton, California, USA). Total phenols were estimated as tannic acid (Sigma, Gillingham, UK) equivalent from the calibration curve produced from a series of dilution of tannic acid solution (0-5 µg tannic acid/ml solution) and the results were expressed as mg/g DM total phenols.

2. 2. 2. 7. 2. Non-tannin phenols

Non-tannin phenols were determined by applying the same steps of total phenols determination (Section 2. 2. 2. 7. 1), after binding the total tannins in the sample extraction to polyvinyl-polypyrrolidone (PVPP, Sigma, Gillingham, UK) as follows: 100 mg PVPP was taken into a 10 ml test tube, 1.0 ml distilled water and 1.0 ml tannins extract (prepared in section 2.2.2.7) were added, respectively. A blank was also prepared similarly with adding 1.0 ml of 70% aqueous acetone instead of tannin extract. After vortexing, the tube was kept in the refrigerator for 15 minutes at 4^{o}C. The tube was vortexed and then centrifuged (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) at 3000 x*g* for 10 minutes at 4^{o}C. 50 µl of the supernatant obtained above was taken and the same steps of total phenol determination (Section 2. 2. 2. 7. 1) were repeated. Total phenols, which simply contain non-tannin phenols rather than tannins, were estimated as tannic acid equivalent from the calibration curve and expressed as mg/g DM.

2. 2. 2. 7. 3. Total tannins

Total tannins (as tannic acid equivalents) were estimated by discarding total phenol readings after treating extraction by PVPP (PVPP, Sigma, Gillingham, UK) from the reading before treating from the following equation.

Equation 2. 1. Total tannin calculation:

$$\text{Total tannins (mg/g)} = \text{Total phenols (mg/g)} - \text{Non} - \text{tannin phenols (mg/g)}$$

2. 2. 2. 7. 4. Condensed tannins

Condensed tannins (proanthocyanidins) were estimated by applying the method of Porter *et al*. (1985) as follows: 200 µl of tannin extract (prepared in section 2.2.2.7), 300 µl of 70% aqueous acetone, 3.0 ml butanol HCl (95:5 v/v) (950 ml 1n-butanol with 50 ml concentrated HCl (37% w/v)) and 100 µl of ferric reagent (2 g ferric ammonium sulphate in 100 ml of 2N HCl) were taken, respectively, into both a 25 ml glass test tube and a 10 ml plastic test tube. The tubes were vortexed. The plastic test tube was left at room temperature (without heating), but the contents of the glass test tube were boiled in a water bath at 100^{o}C for 60 minutes after placing a glass marble on the mouth of the tube. The glass tube was then left to cool down to room temperature. The optical density of the glass tube contents (boiled mixture) was measured at 550 nm after reading plastic test tube contents (without heating) as a blank using a spectrophotometer (Beckman DU 650 spectrophotometer, Beckman instruments, INC. Fullerton, California, USA). Condensed tannins (as leucocyanidin equivalents%) were measured using the following equation.

Equation 2. 2. Condensed tannin calculation:

$$\text{Condensed tannins}\% = \frac{\text{A}_{550\text{ nm}} \times 78.26 \times \text{dilution factor}}{\text{dry matter}\%}$$

When 78.26 is a constant; A is absorbance at 550 nm and dilution factor was 2.5.

2. 2. 2. 8. Grain quality

2. 2. 2. 8. 1. Determination of colour scores

Approximately 200 g of clean representative sample of whole (WB) and fine milled (FMB) bean of each bean cultivar sample was placed in a petri dish, and then was flattened with a ruler. Colour measurements of WB and FMB of each sample were read in triplicate, after submerging the instrument into the samples in petri dishes, employing an L* a* b* colour space (Konica Minolta, Chroma Meter CR-400). The instrument was calibrated against a standard white-coloured reference tile and cleaned between taking measurements of different samples. The L* indicates lightness, 0-100 representing dark to light. The a* value gives the degree of the red-green colour, with a higher positive a* value indicate more red. The b* value indicates the degree of the yellow-blue colour, with a higher positive b* value indicating more yellow.

2. 2. 2. 8. 2. Measuring the weight of 1000 grains

Aprroximately three kg of a representative sample of each field bean variety was taken and thoroughly mixed, exactly 1000 grains were then counted randomly. The weight of

1000 grains was expressed as g DM after determination of DM content of each bean cultivar sample.

2. 2. 2. 8. 3. Measuring water holding capacity

Water holding capacity (WHC) of the field bean samples was measured as follows: An amount of 6 g of a fine milled sample was taken into a 50 ml plastic tube, 15 ml of distilled water was then added and the lead was put back on the tube and placed into a shaking water bath for 2 hours at 40^{o}C. The tube contents were centrifuged (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) at 2200 x*g* for 10 minutes, the liquid portion was then removed, and the tube was drained on paper for 5 minutes. The WHC of the field bean sample was measured as the difference between its dry and wet weight.

2. 2. 2. 8. 4. Measuring water extract viscosity

Water extract viscosity (WEV) of the field bean samples was measured as following. An amount of 2 g of fine milled (passing through a 0.8 mm screen) sample was weighed into a 10 ml plastic tube, 4 ml of deionised distilled water was then added. The field bean sample and water were thoroughly stirred and the tube was then incubated in a 40^{o}C water bath for 30 minutes. Following that, the tube was centrifuged (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) at 10000 x*g* for 2 minutes, and then left at room temperature for 15 minutes, and finally, a 0.5 ml of the supernatant was taken for measuring WEV. The WEV (in centipoise (cP) units) was measured employing a rotating cone and cup viscometer (model DV–II+LV, Brookfield Engineering Laboratories, USA).

2. 2. 2. 8. 5. Measuring seed-coat to cotyledon ratio

One hundred grams of clean representative grain sample of each field bean variety was taken for measuring hull: kennel. Seed-coats were completely separated from cotyledons with the aid of pliers, and the weights of each of cotyledons and seed-coats alone were measured.

2. 2. 3. Statistical analyses

Microsoft Excel 2010 was used for calculations and descriptive statistical analysis. The coefficient of variation (CV%) of the variables was determined in order to express the variability among the experimental cultivars. To detect the relationship between the compositional profile and physical characteristics, correlation coefficients were obtained for all the field bean samples. This was to provide a rapid test to estimate the nutritional value of field bean cultivars to be used by the feed industry. While all measured variables of the field bean samples were included as statistical terms in the analysis, only significant correlations were reported. Correlations were reported as significant at $P<0.05$ ($0.632 \leq r < 0.765$) and $P<0.01$ ($r \geq 0.765$).

2. 3. Results

2. 3. 1. Proximate analysis and gross energy

There were differences in the chemical composition and GE among the studied field bean cultivar samples (Table 2. 1). The overall means of protein, ash, oil and GE of the beans were 282.4, 35.9, 10.8 g/kg DM and 18.46 MJ/kg DM, respectively. Generally, the GE contents were quite similar between different cultivars that ranged from 18.27 (Bazz and Sultan) to 18.60 MJ/kg DM (Divine), indicating a difference of 0.33 MJ, and with a CV 0.7%.

Crude protein concentration was the most variable constituent among bean samples, and the difference between cultivars was approximately 60 g/kg DM, as varied from 244.6 for Sultan to 304.5 g/kg DM for Maris Bead (MB) cultivar. The CP content of MB was just above those of Wizard, Divine (300 g/kg DM) and Honey (294 g/kg DM). The amount of ether extract was greatest in cultivar Fuego, but lowest in cultivar Divine, 13 *versus* 9 g/kg DM (CV=10.1%). The difference in ash among varieties was 7.4 g, as Sultan contained 39.4 g/kg DM, although Arthur contained 32.0 g/kg DM.

The mean of DM concentration was 854 g/kg, and varied from 836 (Honey) to 866 (Divine) g/kg among all bean varieties. With the exception of Buzz (845 g/kg), Honey and Divine, almost similar DM content (about 860 g/kg) was found among the other bean samples.

Table 2. 1. The chemical composition of ten UK grown studied field bean cultivars

Bean cultivar	Dry matter (g/kg)	Ash (g/kg DM)	Oil (g/kg DM)	Protein (g/kg DM)	Gross energy (MJ/kg DM)
Arthur	859	32.0	11.6	270.6	18.41
Buzz	845	38.2	10.7	276.0	18.27
Clipper	854	35.6	9.4	284.8	18.38
Divine	866	38.6	9.2	299.6	18.60
Fuego	855	34.3	12.9	269.8	18.58
Fury	856	33.8	10.5	281.0	18.56
Honey	836	34.7	10.8	293.8	18.56
Maris Bead	858	33.5	10.5	304.5	18.41
Sultan	856	39.4	11.7	244.6	18.27
Wizard	855	38.8	10.5	299.7	18.59
CV%	1.0	7.4	10.1	6.5	0.7

Each value represents mean of duplicate analysis.

2. 3. 2. Amino acids in field beans

The AA profile of the studied field beans expressed as g/kg DM and as g/kg CP is illustrated in Tables 2. 2 and 2. 3. Generally, the highest concentrations (as g/kg DM) for all AAs were found in Divine, while the lowest concentrations for all AAs, except proline and tyrosine, were observed in Sultan cultivar. Honey was the second richest cultivar after Divine, and Clipper was the second poorest cultivar after Sultan for the contents of the majority of AAs.

Amongst the indispensable AAs, the means of arginine (92.2 g/kg CP), leucine (77.6 g/kg CP) and lysine (67.9 g/kg CP) were superior, while those of methionine (7.8 g/kg CP), tryptophan (8.2 g/kg CP) and histidine (27.8 g/kg CP) were inferior compared to those of the other indispensable AAs. Large CVs of 9.4 and 8.8% were noted with tryptophan and arginine, respectively.

Within dispensable AAs, there were high averages of glutamic acid (175.2 g/kg CP) and aspartic acid (116 g/kg CP), but very low averages of cysteine (12.6 g/kg CP), tyrosine (23.3 g/kg CP) and proline (40.2 g/kg CP). High CVs for dispensable amino acids were observed for tyrosine (13.8%), proline (10.8%) and cysteine (10.5%).

Table 2. 2. Amino acid concentrations (g/kg DM) of ten UK grown studied field bean cultivars

Amino acids	Bean cultivar										CV%
	Arthur	Buzz	Clipper	Divine	Fuego	Fury	Honey	Maris Bead	Sultan	Wizard	
Indispensable amino acids											
Arginine	24.0	26.3	22.8	30.3	23.0	27.6	28.1	30.4	19.5	29.5	14.0
Histidine	7.2	8.4	7.1	8.9	6.6	8.4	8.9	8.4	6.4	8.5	11.9
Isoleucine	11.9	13.5	11.1	14.0	11.1	13.0	13.9	12.8	10.2	13.1	10.5
Leucine	21.0	23.6	19.4	24.7	19.5	22.9	24.3	23.2	17.8	23.2	10.8
Lysine	18.9	20.7	17.6	21.0	17.4	20.0	20.7	19.2	16.2	20.0	8.5
Methionine	2.3	2.2	2.1	2.4	2.0	2.3	2.3	2.3	1.8	2.3	9.3
Phenylalanine	14.1	14.4	12.1	14.8	11.5	13.8	14.8	13.4	10.7	14.0	10.8
Threonine	10.2	10.8	9.5	11.8	9.4	11.1	11.4	11.1	8.5	11.0	9.9
Tryptophan	2.4	2.4	2.1	2.9	2.1	2.5	2.4	2.2	1.9	2.3	16.9
Valine	13.3	14.7	12.5	15.4	12.5	14.5	15.1	14.2	11.2	14.3	9.7
Dispensable amino acids											
Alanine	12.2	12.7	10.9	13.9	11.0	13.1	13.3	12.8	9.9	12.7	10.1
Aspartic acid	32.2	34.9	28.9	36.3	29.6	34.8	35.9	34.0	26.3	35.0	10.4
Cysteine	3.4	3.3	3.0	4.4	3.4	3.9	4.1	3.7	2.6	3.9	14.9
Glutamic acid	47.8	52.2	44.2	55.7	44.8	52.4	53.8	52.3	40.3	51.7	10.1
Glycine	12.5	13.1	11.6	14.6	11.6	13.4	13.9	13.4	10.4	13.4	9.9
Proline	8.4	11.7	10.1	13.6	10.9	12.1	12.7	12.8	9.2	12.3	14.9
Serine	13.7	15.0	12.9	16.1	13.1	14.9	15.7	15.3	11.4	15.4	10.4
Tyrosine	5.8	6.7	6.4	8.5	4.4	7.4	7.4	7.0	5.7	6.5	16.9

Each value represents mean of duplicate analysis.

Table 2. 3. Amino acid concentrations (g/kg CP) of ten UK grown studied field bean cultivars

Amino acids	Bean cultivar										CV%
	Arthur	Buzz	Clipper	Divine	Fuego	Fury	Honey	Maris Bead	Sultan	Wizard	
Indispensable amino acids											
Arginine	88.6	95.2	80.2	101.0	85.4	98.1	95.7	99.9	79.8	98.3	8.8
Histidine	26.7	30.5	25.1	29.3	24.3	29.9	30.1	27.6	26.3	28.5	7.9
Isoleucine	43.9	48.9	39.1	46.7	41.2	46.1	47.2	42.1	41.6	43.7	7.1
Leucine	77.4	85.4	68.3	82.5	72.4	81.5	82.6	76.2	72.6	77.3	7.0
Lysine	69.7	75.1	61.7	70.2	64.6	71.1	70.4	63.2	66.4	66.7	6.0
Methionine	8.6	8.2	7.4	8.1	7.4	8.3	7.7	7.7	7.2	7.8	5.8
Phenylalanine	52.0	52.3	42.4	49.3	42.5	49.0	50.5	44.0	43.9	46.8	8.2
Threonine	37.8	39.0	33.3	39.3	34.7	39.5	38.7	36.4	34.9	36.7	5.9
Tryptophan	9.0	8.6	7.4	9.6	7.8	8.7	8.1	7.3	7.6	7.8	9.4
Valine	49.0	53.2	44.0	51.3	46.4	51.5	51.3	46.7	45.9	47.6	6.2
Dispensable amino acids											
Alanine	45.2	45.9	38.3	46.3	40.8	46.5	45.2	42.1	40.6	42.5	6.6
Aspartic acid	119.1	126.5	101.6	121.1	109.7	123.8	122.1	111.8	107.5	116.7	6.9
Cysteine	12.5	12.0	10.7	14.7	12.6	13.7	13.8	12.2	10.5	12.9	10.5
Glutamic acid	176.8	189.2	155.1	185.8	166.1	186.6	183.2	171.9	164.8	172.5	6.4
Glycine	46.0	47.6	40.7	48.6	42.9	47.8	47.2	44.0	42.5	44.9	5.8
Proline	31.0	42.5	35.4	45.5	40.3	43.2	43.2	42.1	37.7	41.0	10.8
Serine	50.8	54.5	45.2	53.6	48.6	53.2	53.3	50.1	46.8	51.5	6.1
Tyrosine	21.5	24.5	22.6	28.5	16.5	26.2	25.2	23.0	23.4	21.9	13.8

Each value represents mean of duplicate analysis.

2. 3. 3. Carbohydrates

The carbohydrate profiles of the field bean samples are displayed in Table 2. 4. The major component of field bean carbohydrates was starch. Its average was 456 g/kg DM among cultivars (CV=7.4%), and ranged from 397 to 517 g/kg DM for cultivars Clipper and Honey, respectively.

Total non-starch polysaccharides and S-NSPs varied from 155.5 and 30.0 (MB) to 250.4 and 72.8 g/kg DM (Clipper), respectively, and I-NSPs differed from 95.9 (Honey) to 177.6 g/kg DM (Clipper). The predominant constituent sugars of T-NSP, in descending order, were glucose, galacturonic acid, arabinose and xylose, whereas, the levels of total galactose and mannose were low and those of both of rhamnose and fucose were scarce. Large CVs were observed for glucose (23.5%), xylose (17.8%) and mannose (17.4%).

The majority of glucose and xylose were detected as insoluble form, but analysed values of both soluble and insoluble arabinose were found almost at the same rate, while the superior portion of galacturonic acid, galactose, rhamnose and fucose was at soluble state. The greatest concentrations of the majority of soluble and insoluble sugars were found in Clipper, while the lowest soluble portions were found in MB and that of insoluble were observed in Honey. Soluble galacturonic acid ranged from 10.1 to 20.3 and glucose from 1.5 to 25 g/kg DM in MB and Clipper, correspondingly, and soluble arabinose scored 7.6 (MB) to 17.6 g/kg DM (Honey). The concentrations of 62.8 to 125.7 of insoluble glucose, and 7.1 to 14.1 g/kg DM of insoluble galacturonic acid were found in Honey and Clipper, respectively. Large CVs were observed with total glucose, soluble and insoluble glucose, mannose, fucose and xylose.

2. 3. 4. Minerals

The mineral content of the studied field bean cultivars is summarised in Table 2. 5. The concentrations of calcium, magnesium, potassium, sodium, sulphur, and boron were similar among the cultivars. Phosphorus concentration varied between 4.33 and 6.87 g/kg DM for Arthur and Wizard, respectively and CV was 15.5%. Copper content was variable between samples, as its concentration in Arthur was in the region of 11.3 mg/g DM lower than that of Buzz, 8.24 *versus* 19.5 mg/g DM (CV=26.3%). The difference of iron was 35 mg/kg DM among cultivar samples, as Clipper had the highest (83.6 mg/g DM), but Honey (49 mg/g DM) the lowest content (CV=16.1%). There was the greatest manganese concentration in Buzz cultivar (31 mg/kg DM) and the lowest (11 mg/kg DM) in Fury and CV was 40.4%. The highest level of zinc was observed in Sultan and the lowest in Arthur cultivar, 64 *versus* 39 mg/g DM (CV=14.1%).

Table 2. 4. Carbohydrate composition (g/kg DM) of ten UK grown studied field bean cultivars

Bean cultivar	Fraction	NSP constituent sugars								Total NSPs	Total starch
		Rha	Fuc	Ara	Xyl	Man	Gal	Glu	GalA		
	Soluble sugar	1.0	0.7	10.2	4.6	2.6	5.3	11.6	14.3	50.3	
Arthur	Insoluble sugar	0.0	0.2	13.4	10.4	2.8	2.8	60.7	8.0	98.3	488
	Total sugar	1.0	0.9	23.5	15.0	5.4	8.1	72.4	22.3	148.6	
	Soluble sugar	0.6	0.4	12.0	5.3	2.2	5.0	10.6	14.5	50.6	
Buzz	Insoluble sugar	0.4	0.6	12.5	14.4	4.1	3.4	91.9	11.9	139.2	452
	Total sugar	1.0	1.0	24.5	19.8	6.2	8.4	102.5	26.4	189.7	
	Soluble sugar	1.3	0.7	10.4	5.8	2.6	6.8	25.0	20.3	72.8	
Clipper	Insoluble sugar	0.0	0.5	13.1	14.6	5.9	3.9	125.7	14.1	177.6	397
	Total sugar	1.3	1.2	23.4	20.3	8.5	10.6	150.7	34.3	250.4	
	Soluble sugar	1.1	0.9	9.6	5.2	2.5	5.8	8.5	12.9	46.4	
Divine	Insoluble sugar	0.0	0.0	11.7	15.0	3.4	3.9	89.2	10.8	134.0	434
	Total sugar	1.1	0.9	21.3	20.2	5.9	9.7	103.6	23.6	180.4	
	Soluble sugar	1.0	0.5	9.7	5.5	2.2	5.0	15.8	14.4	54.1	
Fuego	Insoluble sugar	0.0	0.5	13.1	12.1	4.1	3.8	73.2	10.1	116.9	473
	Total sugar	1.0	1.0	22.9	17.5	6.3	8.8	89.0	24.4	171.0	
	Soluble sugar	1.1	0.8	9.7	5.4	2.1	4.8	5.8	14.5	44.1	
Fury	Insoluble sugar	0.0	0.2	12.1	15.5	4.2	3.5	91.4	9.4	136.4	464
	Total sugar	1.1	1.0	21.8	20.9	6.3	8.4	97.2	23.9	180.5	
	Soluble sugar	1.1	0.7	17.6	5.6	6.9	6.9	7.2	16.9	62.9	
Honey	Insoluble sugar	0.0	0.3	11.1	10.0	2.1	2.6	62.8	7.1	95.9	517
	Total sugar	1.1	1.0	28.7	15.6	8.9	9.5	70.1	23.9	158.8	
	Soluble sugar	0.9	0.7	7.6	2.8	1.4	4.9	1.5	10.1	30.0	
Maris Bead	Insoluble sugar	0.2	0.2	12.5	11.4	4.2	3.3	80.9	12.7	125.5	443
	Total sugar	1.1	0.9	20.1	14.3	5.6	8.2	82.3	22.8	155.5	
	Soluble sugar	1.0	0.4	9.7	3.7	2.1	5.4	15.4	17.1	54.8	
Sultan	Insoluble sugar	0.0	0.5	11.4	8.2	4.6	3.1	96.1	11.6	135.4	467
	Total sugar	1.0	0.9	21.0	11.9	6.6	8.5	111.5	28.7	190.2	
	Soluble sugar	0.8	0.5	11.1	3.6	2.0	5.6	4.9	14.2	42.8	
Wizard	Insoluble sugar	0.3	0.4	11.8	15.8	5.0	3.2	101.8	12.1	150.4	424
	Total sugar	1.2	0.9	23.0	19.5	6.9	8.8	106.7	26.3	193.2	
	Soluble sugar	19.4	27.0	24.8	21.3	57.7	13.4	63.8	18.2	22.9	
CV%	Insoluble sugar	171.4	55.3	6.4	21.0	27.0	12.9	22.1	20.0	18.4	7.4
	Total sugar	9.4	10.5	10.4	17.8	17.4	9.1	23.5	14.0	15.8	

Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glu, glucose; GalA, galacturonic acid; Total-NSPs, total non-starch polysaccharides; Each value represents mean of duplicate analysis.

Table 2. 5. Mineral composition (dry matter basis) of ten UK grown studied field bean cultivars

Beans cultivar	Mineral										
	Calcium (g/kg)	Magnesium (g/kg)	Phosphorus (g/kg)	Potassium (g/kg)	Sodium (g/kg)	Sulphur (g/kg)	Boron (mg/kg)	Copper (mg/kg)	Iron (mg/kg)	Manganese (mg/kg)	Zinc (mg/kg)
Arthur	1.35	1.17	4.33	11.15	0.19	1.89	11.25	8.24	72.95	23.80	39.45
Buzz	1.34	1.41	6.64	12.75	0.12	1.58	10.35	19.50	72.60	31.10	47.55
Clipper	1.60	1.47	6.05	11.10	0.43	1.92	10.55	18.60	83.60	19.55	52.05
Divine	1.18	1.43	5.05	13.70	<0.12	2.92	10.60	12.80	62.30	13.85	45.90
Fuego	1.07	1.34	4.96	11.85	0.30	2.12	11.50	13.90	65.05	11.10	48.65
Fury	1.00	1.17	5.07	11.75	0.31	2.12	11.25	12.75	58.75	10.70	44.70
Honey	0.82	1.37	5.26	11.80	<0.12	2.00	11.40	11.75	48.60	11.25	45.40
Maris Bead	1.00	1.27	5.33	11.20	<0.12	2.08	12.15	16.00	51.40	12.30	53.20
Sultan	1.19	1.42	4.61	13.10	<0.12	1.47	10.65	9.54	67.55	23.60	64.30
Wizard	1.41	1.46	6.87	12.05	<0.12	2.22	10.70	15.95	68.50	15.20	43.85
CV%	19.60	8.3	15.5	7.3	NA*	19.4	5.1	26.3	16.1	40.4	14.1

*NA, not applicable; Each value represents mean of duplicate analysis.

2. 3. 5. Phenols, tannins, phytate and trypsin inhibitors

Total phenols, tannins, non-tannin phenols, condensed tannins, phytate and trypsin inhibitor contents of the studied field bean cultivars are presented in Table 2. 6. The majority of phenolic compounds in the field beans were tannins and non-tannin phenols were low. Amongst the varieties, the total phenol concentrations, as tannic acid equivalents, were the highest in Sultan and lowest in Arthur, 10.9 and 4.5 mg/g DM, correspondingly. Non-tannin phenol contents, as tannic acid equivalents, were low ranging from about 0.8-2.8 mg/g DM amongst samples for MB and Honey, respectively. Among the field bean cultivars, the amount of tannins, as tannic acid equivalents, varied between 2.2 (Buzz) and 8.3 (Sultan) mg/g DM and CV was 34.3%. The mean of condensed tannin (CT) contents, as leucocyanidin equivalents, in bean cultivars was 5.04 mg/g DM (CV=30.9%), and it ranged from 2.8 (Arthur) to 7.3 (Sultan) mg/g DM.

The overall mean of phytate was 14.5 mg/g DM, and the extent of difference among cultivars was up to 11 mg/g DM and CV was 24.6%. The highest phytate concentration was in Buzz (20.84 mg/g DM), while Sultan contained the lowest amount (9.86 mg/g DM).

The average of trypsin inhibitor concentration of the bean samples was 3.5 mg/g DM, and differed from 2.3 (Sultan) to 4.4 (Fuego) mg/g DM with a CV of 19.2%.

Table 2. 6. Total phenols, tannins, non-tannin phenols (NTPH), condensed tannins, phytate and trypsin inhibitor contents (mg/g DM) of ten UK grown studied field bean cultivars

Bean Name	Total phenols[a]	Tannins[a]	NTPH[a]	Condensed tannins[b]	Phytate	Trypsin inhibitors
Arthur	4.5	3.5	1.0	2.8	9.86	3.1
Buzz	4.7	2.2	2.5	2.9	20.84	2.6
Clipper	7.1	4.6	2.5	5.3	16.62	3.3
Divine	7.1	4.8	2.4	6.2	13.35	4.2
Fuego	8.3	6.1	2.3	6.8	12.90	4.4
Fury	6.3	4.3	2.0	4.7	13.77	3.7
Honey	7.3	4.4	2.8	3.9	13.51	3.4
Maris Bead	6.9	6.1	0.8	4.5	13.90	3.8
Sultan	10.9	8.3	2.6	7.3	10.63	2.3
Wizard	8.1	6.8	1.4	6	19.80	3.8
CV%	25.7	34.3	35.0	30.9	24.6	19.2

[a]As tannic acid equivalents; [b]As leukocyanidin equivalents. Each value represents mean of triplicate analysis.

2. 3. 6. Colour scores

Colour score of whole field bean seed (WB) and bean flour (FB) is illustrated in Table 2. 7. Lightness of whole bean (L* WB) ranged from about 34.8 (Buzz) to 52.5 (Fury). There was a similar L* WB of almost 52 for each of Divine, Fuego, Fury and MB, followed by Wizard (51) and then Honey 48. Also, L* WB scores of Sultan, Arthur, Buzz and Clipper were low and similar (about 35-37). The lightness score of bean flour (L* FB) differ from about 88 for Sultan to 95 for Fury, and the CV was 2.4%. The redness-greenness degree of whole bean (a* WB) varied from 8 for Honey to 14 for Sultan and CV% was 15.4. Also, redness-greenness degree of bean flour (a* FB) was different from 0.99 to 1.44, for Divine and Sultan, respectively. The mean of yellowness-blueness degree of whole bean (b* WB) was 20. The highest score was 26 for MB and the lowest was about 14 for Buzz and Arthur. The overall mean of yellowness-blueness degree of bean flour (b* FB) was about 18 and CV% was 10.6, as Sultan cultivar had the highest value of 22, while Buzz cultivar had the lowest value of 15.

Table 2. 7. Colour score (L*, a* and b*) and of whole (WB) and flour (FB) of ten UK grown field bean cultivars*

Bean Name	L* WB	L* FB	a* WB	a* FB	b* WB	b* FB
Arthur	35.71	93.59	12.53	1.07	14.37	17.72
Buzz	34.77	91.49	11.78	1.27	14.10	14.69
Clipper	37.25	91.76	10.97	1.17	15.58	18.94
Divine	52.49	94.66	9.70	0.99	22.95	17.59
Fuego	52.45	94.25	9.84	1.14	22.72	17.96
Fury	52.51	95.16	10.68	1.21	23.20	18.22
Honey	48.48	94.63	8.02	1.06	19.69	17.04
Maris Bead	52.22	93.18	12.03	1.01	26.06	19.05
Sultan	36.03	87.71	14.21	1.44	15.45	22.29
Wizard	51.08	94.04	11.51	1.18	23.62	19.34
CV%	18.0	2.4	15.4	11.7	22.8	10.6

* L* WB, lightness-darkness degree of whole bean; L* FB, lightness-darkness degree of bean flour; a* WB, redness-greenness degree of whole bean; a* FB, redness-greenness degree of bean flour; b* WB, yellowness-blueness degree of whole bean; b* FB, yellowness-blueness degree of bean flour; Each value represents mean of triplicate analysis.

2. 3. 7. Weight of 1000 grains, water holding capacity, water extract viscosity and cotyledon to seed-coat ratio

Thousand-grain weight (TGW), water holding capacity (WHC), water extract viscosity (WEV), cotyledon ratio and seed-coat ratio of the characterised field bean samples are summarised in Table 2. 8. The mean of WHC of the field bean samples was 1117 g/kg DM, and the variation among cultivars was 149 g and CV was 4.4%. The highest WHC of

1180 g/kg DM was reported with Fury, which was similar to that of Fuego and Sultan. Buzz had the lowest WHC capacity of 1031 g.

Regarding TGW, the overall mean was 546 g DM. Honey grains were the heaviest, which were more than twice that of MB, 754 *versus* 311g. All of Buzz, Arthur and Wizard had almost the same grain weight of approximately 0.7 g/grain, followed by Clipper and Fury and Fuego of about 0.5 g/grain, whereas, both Divine and Sultan owned about 0.4 g grain weight. Water extract viscosity of field beans was variable (CV=25.8%), ranging from 2.07 (Arthur) to 5.01 (MB) cP. The average of seed-coat proportion was 136 g/kg and CV was 10.1%. Clipper and then Sultan had the highest proportion of 157 and 156 g/kg, respectively, although Honey had the lowest seed-coat ratio of 110 then MB of 123 g/kg.

Table 2. 8. Weight of 1000 grains (TGW), water holding capacity (WHC), water extract viscosity (WEV), and cotyledon and seed-coat ratio of ten UK grown studied field bean cultivars

Bean cultivar	TGW (g DM)	WHC (g/kg)	WHC (g/kg DM)	WEV (cP)	Cotyledon (g/kg)	Seed-coat (g/kg)
Arthur	685	915	1065	2.07	866.4	133.6
Buzz	693	871	1031	2.41	868.9	131.1
Clipper	539	943	1104	4.52	843.4	156.6
Divine	444	935	1080	4.18	863.2	136.8
Fuego	466	1005	1175	3.58	858.1	141.9
Fury	483	1010	1180	4.59	863.7	136.3
Honey	754	956	1144	4.81	889.8	110.2
Maris Bead	311	961	1120	5.01	876.7	123.3
Sultan	407	997	1165	4.04	844.5	155.5
Wizard	681	947	1108	3.40	867.3	132.7
CV%	27.1	4.5	4.4	25.8	1.6	10.1

Each value of WHC and WEV represents mean of triplicate analysis

2. 3. 8. Correlation coefficients (*r*) between different measured variables studied in the field bean cultivars

Gross energy content in the studied field beans had positive (P<0.01) relationship with L* FB (r=0.854), L* WB (r=0.852) and sulphur content (r=0.800). Crude protein concentration in field beans was positively correlated (P<0.05) to L* FB (r=0.683), b* WB (r=0.637), sulphur content (r=0.674), but negatively correlated (P<0.01; r=-0.772) to a* FB. Total starch level was negatively correlated (P<0.01) to T-NSPs (r=-0.756) and total I-NSPs (r=-0.916) in the beans. There was a significant (P<0.05) positive correlation between cotyledon ratio and the content of indispensable (r=0.683), dispensable (r=0.650) and total (r=0.668) AAs, arginine (r=0.734), histidine (r=0.641) and threonine (r=0.647) (expressed as g/kg CP) in the field beans. Also, a negative relationship (P<0.05) between each of the indispensable (r=-0.662), total amino acid (r=-0.643), lysine (r=-0.640) and methionine (r=-

0.663) contents (expressed as g/kg CP) and b* FB was detected. Moreover, soluble glucose, which had a negative correlation with cotyledon ratio (P<0.05; r=-0.759), also had a negative correlation (P<0.05) with indispensable (r=-0.680), dispensable (r=-0.677), total amino acids (r=-0.681), histidine (r=-0.667) and arginine (P<0.01; r=-0.883) in the studied field beans.

There was a negative correlation (P<0.05) between the cotyledon ratio and total I-NSP (r=-0.643), T-NSP (r=-0.687), iron (r=-0.738), soluble glucose (r=-0.759), insoluble glucose (r=-0.664), total galacturonic acid (r=-0.699) and total glucose (P<0.01; r=-0.776) in the field beans.

A negative correlation (P<0.05) was found between the contents of condensed tannin and both methionine (r=-0.708) and phenylalanine (r=-0.725) in field beans. Similarly, total tannins had a negative correlation (P<0.05) with methionine (r=-0.717) phenylalanine (r=-0.717) and valine (r=-0.655) levels in the field beans. The concentration of both total tannins (r=0.919) and condensed tannins (r=0.888) were highly positively correlated (P<0.001) to that of total phenols in the beans.

Phytate had a positive relationship (P<0.01) with phosphorus (r=0.984), copper (r=0.871) and insoluble xylose (P<0.05; r=0.688). A positive relationship between phosphorus and copper (P<0.001; r=0.849) and insoluble xylose (P<0.05; r=0.641) was noted. There was also a positive relationship (P<0.05) between sulphur content, L* WB (r=0.733) and L* FB (r=0.712), while manganese content was negatively correlated to L* WB (P<0.01; r=-0.912) and L* FB (P<0.05; r=-0.669) in beans. Additionally, L* WB had a negative relationship (P<0.05; r=-0.676) with iron concentration in the studied bean cultivars.

2. 4. Discussion

The purpose of this study was to investigate the chemical composition and physical characteristics of ten different UK grown field bean cultivars and to find out the differences between them. Variations among the cultivars were expressed using CV values. The dissimilarity in the chemical composition physical characteristic results among those investigated in this study and different cultivars reported in the literature may be explained by differences in genotype (Marquardt *et al.,* 1975; Makkar *et al.,* 1997; Duc *et al.,* 1999; Jezierny *et al.,* 2011; Oomah *et al.,* 2011), growing season, location (Marquardt *et al.,* 1975; Bond, 1976; Duc *et al.,* 1999; Oomah *et al.,* 2011), and the interaction of genotype and environmental factors.

Gross energy values were very close among the samples, and were within a similar range to those reported by other authors for many European grown field bean cultivars (Makkar *et al.,* 1997; Duc *et al.,* 1999; Grosjean *et al.,* 2001). Regarding CP concentrations, Sultan cultivar contained a low amount (245 g/kg DM), but the observed values for the rest of the cultivars were in the range previously documented in the literature (Bond, 1976; Makkar *et al.,* 1997; Brufau *et al.,* 1998; Duc *et al.,* 1999; Usayran *et al.,* 2014). The low

concentration of CP, in combination with a high seed-coat ratio found in the Sultan cultivar compared to the other field bean cultivars was probably due to Sultan having the highest concentration of tannins. It has been found that lower levels of CP in tannin-containing winter-grown field bean cultivars is compensated by higher fibre rather than starch (Duc *et al.,* 1999). It has also been widely reported that high tannin containing field bean cultivars contain lower levels of CP than low tannin ones (Makkar *et al.,* 1997; Jezierny *et al.,* 2011; O'Neill *et al.,* 2012). The oil concentrations in the studied field bean cultivars in this assay were in the expected range (Marquardt *et al.,* 1975; Usayran *et al.,* 2014). The ash content of the analysed field bean samples in this study fall within the range documented earlier for many different field bean cultivars (Marquardt *et al.,* 1975; Makkar *et al.,* 1997; Duc *et al.,* 1999; Usayran *et al.,* 2014).

Moderately high CV values were only noticed for cysteine, tryptophan, proline and tyrosine. The AA profile of the Sultan cultivar was in agreement with those of field beans documented by Nalle *et al.* (2010a), and the AA profile of nine other varieties was similar to those earlier reported for many field bean cultivars (Makkar *et al.,* 1997; Duc *et al.,* 1999; Jezierny *et al.,* 2011; O'Neill *et al.,* 2012; Usayran *et al.,* 2014). The lower concentration of most AAs in Sultan compared with those of other cultivars is connected to the low CP level in this sample, compared to that of others, as a high positive relation between the concentration of AAs and CP in field beans has been observed (Mossé and Baudet, 1977; Bjerg *et al.,* 1988; Duc *et al.,* 1999). These results showed that AA contents are negatively correlated to seed-coat ratio of field beans. In conformation of this, Sultan and Clipper had the highest seed-coat ratio and therefore the lowest concentration of AAs. Conversly, Honey had the lowest seed-coat proportion and was the second richest cultivar in AAs (after Divine). Furthermore, MB had the second lowest seed-coat ratio and was the third richest cultivar in most AAs, compaired to the other field bean cultivars.

With the exception of Honey, which had a high concentration of total starch (517 g/kg DM), the rest of bean samples were within the ranges reported by Makkar *et al.* (1997) and Duc *et al.* (1999). The highest concentration of starch in Honey was coupled with the highest seed weight and the lowest seed-coat ratio in this cultivar amongst the studied cultivars, thus indicating the carbohydrate has been stored as starch rather than NSPs. In agreement with the findings of the current study, a positive correlation between the seed-coat ratio and the level of fibres and negative correlation between seed-coat ratio and starch in field beans has been noted earlier (Marquardt *et al.,* 1975).

Total non-starch polysaccharide, I-NSP and S-NSP levels in the analysed samples in this study were comparable to those documented by Rubio *et al.* (1992), Nalle *et al.* (2010a) and Adamidou *et al.* (2011) for the relevent field bean cultivars. Regarding the concentration of constituent sugars of NSPs in these field bean cultivars and their soluble and insoluble segments, results were comparable to those documented for field beans in the literature (Rubio *et al.,* 1992; Perez-Maldonado *et al.,* 1999). The concentration of T-

NSPs (250 g/kg DM) in Clipper was the highest and starch was much lower than that of the rest of samples, this was associated with the highest seed-coat ratio in this cultivar among the samples. Non-starch polysaccharide contents are positively associated with seed-coat ratio in this study. Moreover, the lowest NSPs in both Honey and then MB among the cultivars were in line with the lowest seed-coat in the former followed by later cultivar among all samples. Marquardt *et al.* (1975) and Duc *et al.* (1999) also reported high positive correlation between fibre contents and seed-coat ratio in field beans.

There were some small variations in the mineral content among cultivars and high variations were only found for copper, iron, zinc and manganese. Makkar *et al.* (1997) reported similar variation among twelve German-grown field bean cultivars. Generally, the concentrations of all of the macro and microelements of these field bean samples fell in the range reported by Makkar *et al.* (1997). Close concentrations of minerals among many white and colour-flowering (Makkar *et al.,* 1997), also winter and spring-grown (Eden, 1968) field bean cultivars have been observed previously.

The content of anti-nutrients including total phenols, total tannins, non-tannin phenols, condensed tannins and trypsin inhibitors of these field beans were within the range reported earlier amongst European cultivars (Makkar *et al.,* 1997). Futhermore, the range of tannins in the current field bean cultivars were well agreed with those detected among many field bean varieties by Duc *et al.* (1999). Differences in phenols, tannins and condensed tannins in field beans are highly related with genotype (Makkar *et al.,* 1997; Duc *et al.,* 1999; Oomah *et al.,* 2011) and to an extent to growing location and genotype by environment interaction (Oomah *et al.,* 2011). The trypsin inhibitor concentrations detected in the current samples were low and within the expected range (Duc *et al.,* 1999). These concentrations of trypsin inhibitors are lower than that in commercially available heat-treated SBM products, which are widely used in poultry diet formulation, thus do not cause physical problems and their impact is negligible for poultry.

The range of phytate levels in these field beans was comparable with that noted amongst various field bean varieties by Makkar *et al*. (1997) and Oomah *et al.* (2011). The L* WB, a* WB and b* WB values found for the field bean cultivars investigated in this study were higher than those documented by Oomah *et al.* (2011) amongst many field bean cultivars who used a different measuring instrument than that used in this study. With the exception of the Sultan cultivar flour, which had almost similar L* FB to that of a pea cultivar (84) (Maninder *et al.,* 2007), and desi and kabuli chickpea cultivars (85) (Kaur and Singh, 2005), all other field bean samples had only approximately 5-10 degrees less L* FB than the aforementioned. Furthermore, with the exception of Sultan flour, which had a slightly higher b* FB value, the values for other field bean samples almost fell within the ranges reported earlier for the flours of chickpea (Kaur and Singh, 2005), pea and pigeon peas (Maninder *et al.,* 2007). The a* FB values of these field bean samples were only one degree higher than those of chickpea and pea flour documented by Kaur and Singh

(2005) and Maninder *et al.* (2007). The differences between L*, a* and b* scores of these field bean cultivars and those in the literature may be related to the type of legume seed, cultivar, growing location and the employed instruments. High L* FB and L* WB and b* WB was coupled with high CP and AA concentrations in this study.

The difference in WHC among samples relates to variance in terms of their polysaccharide content, particularly acid detergent fibres (Elhardallou and Walker, 1993). With the exception of TGW in the MB sample (311 g), the values for all other cultivars were in the ranges previously reported for UK grown (Bond, 1976) faba beans. In addition, excluding MB and Honey cultivars, the TGW of others fell in the range previously documented for field beans by Grosjean *et al.* (2001) and Oomah *et al.* (2011). The dissimilarity of seed weight of MB and Honey with those documented in the literature is related to the very small size (0.311 g/seed) of the former and large size (0.754 g/seed) of the later cultivars. According to Oomah *et al.* (2011), who investigated 13 field bean cultivars grown in two different locations, seed weight is highly connected with genotype and growing location, as well as interactions between genotype and growing location.

The range of seed-coat ratio of these cultivars was in agreement with those reported for field beans in the literature (Bond, 1976; Brufau *et al.,* 1998; Duc *et al.,* 1999). There is a connection between seed-coat ratio and tannins in field beans. The highest seed-coat in Sultan comparing with the other cultivars agreed with the highest phenols, tannins and condensed tannins in this cultivar compared to the rest of samples. In addition, 2.1% (Duc *et al.,* 1999) and 2.8% (Helsper *et al.,* 1993) higher seed-coat proportion have been reported in high tannin over low tannin-field bean cultivars.

2. 5. Conclusion

The data displays that the studied field bean cultivars are different in terms of their chemical composition and physical characteristics. The experimental field bean samples were winter and spring grown cultivars, and were obtained from different UK farms. Therefore, the differences in the chemical composition and physical characteristics of the studied field bean samples were possibly due to environmental factors, such as growing location, climate and gowing season, and not specifically due to cultivar. Quite high variations were found for a few amino acid concentrations such as cysteine, tryptophan, proline and tyrosine, and a few minerals such as manganese, copper, iron and zinc, and weight of bean seeds and seed-coat ratio. Additionally, large variations were observed among cultivars in terms of their NSPs, phenols, tannins, condensed tannins and phytate contents. According to researchers, the differences in the compositional profile of field beans relate to genotype, climate under which they are grown, growing location, soil quality and interaction of genotype with environmental factors.

Generally, the data from the current study shows that these field bean cultivars are typical and their nutrient and anti-nutrient contents are consistent with those documented for

European cultivars. Nevertheless, of the variabilities measured in the composition of the current field bean cultivars, starch forms the majority of the carbohydrate content. Other nutrients (especially CP and AAs) were also high and the AA profile (expressed as g/kg CP) of these samples is comparable to that of SBM. Thereby, these field bean cultivars can be utilised as an acceptable source of CP and AAs for poultry diet formulation, particularly when threonine, methionine, cysteine and tryptophan are supplemented. Following on from this study, a growing broiler feeding experiment is needed to determine the inherent energy and nutrient bioavailability of the studied field bean cultivars; relating this to the determined parameters in this study.

CHAPTER 3: ENERGY AND NUTRIENT UTILISATION IN FIELD BEANS FOR BROILER CHICKENS

3. 1. Introduction

Field bean cultivars are different in terms of their nutritive value for broiler chickens, depending on their chemical composition. Currently, there is no information about the energy and nutrient availability of the UK field bean cultivars characterised in the previous chapter. The main objectives of this experiment, therefore, were to examine the differences in metabolisable energy and nutrient utilisation of the 10 field bean cultivar samples described in the previous chapter when fed to broilers. The aim was also to examine the relationship between the chemical composition of the field bean samples and the bioavailability of energy and nutrients.

3. 2. Materials and methods

3. 2. 1. Field bean samples

The ten field bean samples characterised in the previous chapter were examined in this feeding trial. All harvested field bean samples were stored at ambient air temperature in a dry store and were used in a broiler feeding experiment after approximately 6 months of storage. Before the animal feeding experiments, the field bean samples were hammer-milled using a 4 mm screen and then mixed in a horizontal mixer with the other feed ingredients. Freshly milled field beans were used in the feeding study to avoid spoilage.

3. 2. 2. Diet formulation

A wheat-soybean meal based balancer diet (control diet) was formulated that had major ingredients of 533.2 g/kg wheat, 150.0 g/kg SBM, 175.0 g/kg full fat soy meal, 37.4 g/kg maize gluten meal, and 50 g/kg soy oil, and contained 231 g/kg CP and 13.71 MJ/kg ME (Table 3. 1). The balancer diet had a higher ME content than breeder's recommendation (Aviagen Ltd., Edinburgh, UK) to allow the ME of the field bean containing diets to be close to the requirements. Ten diets were then produced including 200 g/kg of one of the ten different field bean cultivars and 800 g/kg of the balancer feed (Table 3. 1). To allow testing of linear response to dietary bioavailable energy and nutrient utilisation of bean samples, another diet was formulated that contained 100 g/kg of the Honey field bean sample and 900 g/kg of the balancer feed, resulting in twelve experimental diets in total. Freshly milled field beans were used in the formulation of the diets and were fed as mash. All diets approximately met or exceeded the dietary specifications for Ross 308 broilers (Aviagen Limited, Edinburgh, UK). Diets did not contain any coccidiostat, antimicrobial growth promoters, prophylactic or other similar additives. To determine marker based

digestibility of fat, DM and AAs in the ileum, all diets were supplied with 5 g/kg of titanium dioxide (TiO_2) as an indigestible marker.

Table 3. 1. Approximate chemical composition and ingredients (g/kg, as-fed) of the experimental diets and as-hatched nutrition specification for Ross 308 broilers

Ingredient	Balancer diet (g/kg)	Diet with field beans (g/kg)
Wheat	533.2	426.6
SBM (CP=48%)	150	120
Full fat soybean meal	175	140
Maize gluten meal	37.4	30
Field beans	-	200
Soy oil	50	40
Lysine	1.9	1.5
Methionine	6.3	5
Threonine	1.9	1.5
Monocalcium phosphate	20	16
Limestone	15.5	12.4
Sodium cloride	3.8	3
Vitamin/mineral premix*	5	4
Total	1000	1000
Determined composition		**
ME (MJ/kg)	13.71	13.17
Protein (g/kg)	231	227-237
Lysine (g/kg)	12.4	12.7-13.6
Methionine + cysteine (g/kg)	11.1	9.6-10.1
Calcium (g/kg)	11.1	9.0-9.2
Available phosphorus (g/kg)	8.5	7.5-8.0
Sodium (g/kg)	2.0	1.6-1.7

*The vitamin and mineral premix contained vitamins and trace elements to meet breeder's recommendation (Aviagen Ltd., Edinburgh, UK). The vitamin and mineral premix provided per kg diet: 50 mg nicotinic acid, 34 mg α-tocopherol, 15 mg pantothenic acid, 7 mg riboflavin, 5 mg pyridoxine, 3.6 mg retinol, 3 mg menadione, 2 mg thiamine, 1 mg folic acid, 200 *μ*g biotin, 125 *μ*g cholecalciferol, 15 *μ*g cobalamin, 100 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 1 mg iodine, 0.5 mg cobalt, 0.5 mg molybdenum and 0.2 mg selenium.
**The calculated average composition of the diet containing field beans was based on NRC (1994) values for field bean ME and available phosphorus.

3. 2. 3. Bird husbandry, experimental design and sample collection

The experiment was approved by the Research Ethics Committee at Harper Adams University.

Approximately five-hundred day old male Ross 308 broiler chicks were obtained from a commercial hatchery (Cyril Bason Ltd, Bank House, Corvedale Road, Craven Arms, Shropshire, SY7 9NG). All chicks were placed in a single rear pen at 33°C and fed a proprietary broiler starter feed *ad libitum* over seven days. On the first day of the experiment (8 days of age), all chicks were individually weighed and outliers were

discarded, leaving 480 birds. Five birds were randomly allocated to one of 96 pens within eight random blocks (0.36 m^2 solid floor area). The pens were arranged in one tier level within a controlled environment and each pen was equipped with a feeder and drinker. The floor of each pen was covered with wood shavings. Each of the twelve experimental diets was allocated at random to 8 pens. Feed and water were provided *ad libitum* throughout the experimental period.

The temperature was gradually reduced daily until room temperature reached 23°C when birds were 21 days old. A standard lighting programme for broilers was used, decreasing from 23:1 (hours light: dark) from zero day old to 18 hours: 6 hours at 7 days of age, which was maintained until the end of the study.

The experiment ended when the birds were 21 days of age. The birds were group-weighed on a per pen basis at the beginning (8 days old) and at the end of the study (21 days old). The average daily feed intake (FI), daily weight gain (WG) and feed conversion ratio (FCR) were determined over this time.

At the beginning of day 18, the solid floor of each pen was replaced with a wire mesh and all excreta were quantitatively collected daily in a plastic tray over the four final days of the experiment, from 18 to 21 days of age. Feed intakes were also measured for the same period. The freshly collected total excreta output of each pen were immediately dried in a forced draft oven at 60°C until a constant weight and then left at room temperature for three days, followed by weighing.

At the end of the study, all birds in each individual pen were killed by cervical dislocation and ileal digesta were collected, pooled on a per pen basis, into an airtight container and frozen at -20^{o}C, and then freeze dried and kept in an airtight container in a fridge. The dried excreta, ileal digeta, as well as representative balancer diet samples were ground to pass through 0.8 mm screen. The DM, GE, nitrogen and fat of each dried excreta and the balancer diet samples were determined in duplicate, as described for the field bean samples earlier (see Secction 2. 2. 2. 1). The concentration of AAs and fat in the digesta and balancer diet samples was also measured using the same procedure for that of the field bean samples (see Secction 2. 2. 2. 2). Titanium concentration in the balancer diet and digesta samples was determined using the method described by Short *et al.* (1996).

The apparent metabolisable energy (AME) and the nitrogen-corrected AME (AMEn) of the diets were calculated as described by Hill and Anderson (1958). The coefficients of total tract fat digestibility (FD), nitrogen (NR) and dry matter (DMR) retention were determined as the difference between intake and excretion of each nutrient divided by its respective intake. The values of dietary apparent ileal AA (AID), ileal fat (IFD) and ileal dry matter (DMD) digestibility coefficients were also determined as described by Lammers *et al.* (2008) and Nalle (2009). After finding a linear response between the field bean inclusion rate and the dietary energy and nutrient availability, the values of energy and nutrient utilisation obtained from only the field beans (excluding those originating from the

balancer feed) was calculated using the slope ratio method described by Finney (1978) and Nalle (2009).

Equation 3. 1. AME calculation:

$$\text{AME}_{\text{ whole diet}}\ (\text{MJ/kg DM}) = \frac{\left(\text{GE}_{\text{ intake}}(\text{MJ}) - \text{GE}_{\text{ output}}\ (\text{MJ})\right)}{\text{Feed intake (kg DM)}}$$

$$\text{AME}_{\text{ field bean}}\ (\text{MJ/kg DM}) = \frac{\text{AME}_{\text{ whole field bean diet}} - (\text{AME}_{\text{ balancer diet}} \times 0.8)}{0.2}$$

Equation 3. 2. AMEn calculation:

$$\text{AMEn}\ (\text{MJ/kg DM}) = \frac{\left(\text{GE}_{\text{ intake}}(\text{MJ}) - \text{GE}_{\text{ output}}\ (\text{MJ})\right) - \left(\text{N}_{\text{ retained}}\ (\text{kg}) \times 34.39\ \text{MJ/kg}\right)}{\text{Feed intake (kg DM)}}$$

$$\text{AMEn}_{\text{ field bean}}\ (\text{MJ/kg DM}) = \frac{\text{AMEn}_{\text{ whole field bean diet}} - (\text{AMEn}_{\text{ balancer diet}} \times 0.8)}{0.2}$$

Apparent metabolisable energy values were corrected for zero nitrogen balance (AMEn) by using a factor of 34.39 KJ per g nitrogen retained in the body (Hill and Anderson, 1958).

Equation 3. 3. NR, FD and DMR calculations:

$$\text{NR}_{\text{ whole diet}} = \frac{\text{N}_{\text{ intake}} - \text{N}_{\text{ output}}}{\text{N}_{\text{ intake}}}$$

$$\text{FD}_{\text{ whole diet}} = \frac{\text{Fat}_{\text{ intake}} - \text{Fat}_{\text{ output}}}{\text{Fat}_{\text{ intake}}}$$

$$\text{DMR}_{\text{ whole diet}} = \frac{\text{DM}_{\text{ intake}} - \text{DM}_{\text{ output}}}{\text{DM}_{\text{ intake}}}$$

Equation 3. 4. DMD, AID of AAs and IFD calculations:

$$\text{DMD}_{\text{whole diet}} = \frac{\text{Ti}_{\text{ ilial digesta}} - \text{Ti}_{\text{ feed}}}{\text{Ti}_{\text{ ilial digesta}}}$$

$$\text{AID of AAs and IFD}_{\text{ whole diet}} = \frac{(\text{X/Ti})\ \text{f} - (\text{X/Ti})\ \text{d}}{(\text{X/Ti})\ \text{f}}$$

Where, (X/Ti) f = ratio of amino acids and fat to titanium in feed; (X/Ti) d = ratio of amino acids and fat to titanium in ileal digesta; Ti = titanium concentration.

Equation 3. 5. Calculation of nutrient availability coefficients of field beans:

$$\text{Field bean nutrient availability coefficient} = \frac{(\text{Nutrient availabilty of field bean diet} \times \text{Nutrient in field bean diet}) - (\text{Nutrient availability of balancer diet} \times 0.8 \times \text{Nutrient in balancer diet})}{0.2 \times \text{nutrient in the field bean alone}}$$

Where, 0.8 and 0.2 are the balancer feed and field bean inclusion rates, respectively, in the diets; nutrient availability is NR, AID of AAs, FD, IFD, DMR and DMD.

3. 2. 4. Statistical analyses

The replicate unit was the cage. Statistical analyses were performed using the Genstat XVII statistical software package (Genstat 17 release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). Regression analysis was used in order to test linear response of bioavailable energy and nutrient utilisation to inclusion rates of the Honey field bean sample. Then the AME, AMEn and values of the nutrient utilisation coefficients were obtained using the slope ratio method (Finney, 1978; Nalle, 2009). The bioavailable energy concentrations and the nutrient utilisation coefficients of the experimental field bean samples were statistically annalysed using a one-way ANOVA in a randomised block design (ten field bean cultivars in eight blocks). Tukey's range test was used to determine significant differences between field bean treatment groups. In all instances, differences were reported as significant at $P \leq 0.05$. Tendencies towards significance were also reported at $P \leq 0.10$.

The coefficients of correlation were obtained for all chemical compositions and quality measurements of the field bean samples. Correlations were reported as significant at $P<0.05$ ($0.632 \leq r < 0.765$) and $P<0.01$ ($r \geq 0.765$). Multiple linear regression analysis was used to assess the relationship between determined bioavailable energy and amino acid digestibility and the laboratory measurements of the bean samples. A step-wise regression technique selected the terms to add as explanatory variables into a linear model. Since AME and AA digestibility are the most important variables required for broiler feed formulation, the determined AME and AA digestibility were used separately as the dependent variables. All measured chemical and physical characteristics of the field bean samples were offered as terms in the multiple linear regressions.

3. 3. Results

The data from the balancer feed and the diet containing 10% Honey field bean cultivar was used to test linearity only (reported in appendices) and was not presented in tables of bean only data. This was due to the indication of a linear relationship between the

inclusion level of field beans and dietary energy and nutrient availability. The data of the SBM-based balancer diet (without added field beans) was used for calculating energy and nutrient bioavailability of field beans as the only ration component for broilers by using a substitutional method or the slope-ratio method (extrapolation) and excluding the values originating from the balancer proportion.

3. 3. 1. Linearity between field bean inclusion rate and dietary energy and nutrient utilisation

There was a linear response to the inclusion rate of Honey bean sample for AME, AMEn, DMR (P<0.001) (Table 3. 2). There was no linear or quadratic response (P>0.05) for NR, FD, IFD and DMD, thus allowing the slope-ratio method to be used for the determination of these variables in the entire field bean cultivar samples.

Table 3. 2. Linearity between field bean (Honey cultivar) inclusion rate and dietary energy and nutrient utilisation

Variable	Field bean inclusion rate in the diets			SEM	*P* value		
	0.0%	10.0%	20.0%		Treatment	Linear	Quadratic
Total collection							
AME (MJ/kg DM)	15.15	14.75	14.20	0.098	<0.001	<0.001	0.547
AMEn (MJ/kg DM)	14.27	13.86	13.30	0.096	<0.001	<0.001	0.540
NR	0.625	0.623	0.621	0.0033	0.697	0.404	0.998
FD	0.749	0.756	0.771	0.0110	0.400	0.193	0.779
DMR	0.716	0.702	0.683	0.0048	<0.001	<0.001	0.644
Ileal digesta digestibility							
IFD	0.876	0.858	0.866	0.0096	0.459	0.465	0.315
DMD	0.644	0.634	0.624	0.0113	0.478	0.233	0.973

3. 3. 2. Broiler growth performance, available energy and nutrient utilisation of field beans

Since the aim of this study was to compare the impact of dietary inclusion rate of 200 g/kg of field bean cultivar samples on broiler performance, the results from the control and 100 g/kg Honey containing diets are not presented but can be found in Appendix 3.

The variation in daily feed intake (FI), daily weight gain (WG) and feed conversion ratio (FCR) was in the expected range for broiler chickens reared from 7 to 21 days old (CV=5.3%, 6.0%, and 2.5%, respectively) (Table 3. 3). The overall final body weight of the birds fed 200 g/kg field bean-containing mash diets was 771 g (not presented in the table),

which was approximately 20% lower than the breeders' recommendation (Aviagen Ltd., Edinburgh, UK) when fed a pelleted diet (959 g at 21 day).

Compared to the breeder's recommendation, FI was over 10 g lower. Although there were no significant differences (P>0.05) in FI, birds fed Divine tended (P=0.060) to grow better than the rest. The overall FCR was in the expected range (Aviagen Ltd., Edinburgh, UK), as the Divine based diet resulted in a better (P<0.001) FCR compared to the Buzz and Sultan base diets, but did not differ (P>0.05) from the rest.

Table 3. 3. Performance of broiler chickens fed on diets containing 200 g/kg of one of the ten different UK grown field bean cultivar samples

Diet	FI (g DM/b/d)	WG (g/b/d)	FCR (g:g)
Arthur	57.4	46.2	1.244^{abc}
Buzz	59.3	47.3	1.254^{bc}
Clipper	58.4	47.1	1.240^{abc}
Divine	60.0	50.0	1.201^{a}
Fuego	57.7	46.9	1.233^{abc}
Fury	58.5	48.2	1.212^{ab}
Honey	58.8	48.3	1.220^{ab}
Maris Bead	59.5	48.7	1.221^{ab}
Sultan	57.4	45.1	1.274^{c}
Wizard	56.5	46.4	1.217^{ab}
Mean	58.3	47.4	1.232
CV%	5.3	6.0	2.5
SEM (df=63)	1.10	1.01	0.0110
P value	0.455	0.060	<0.001

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; Each value represents mean of eight replicate pens of five birds each; Bird performance was determined from 7 to 21 days of age; CV, coefficient of variation; SEM, standard error of the mean; ^{a,b,c}Values within a column with different superscripts differ significantly at P≤0.05.

The results based on ileal digesta including IFD and DMD are presented in Table 3. 4. Although the IFD coefficients of the studied field bean cultivars did not differ (P>0.05), there were differences (P<0.05) in their DMD. Coefficients of ileal dry matter digestibility varied from 0.345 (Sultan) to 0.543 (Honey) (CV=22.6%). Sultan cultivar had a lower (P<0.05) DMD value than that of Honey, Wizard and Fuego, while the DMD content of these four bean samples did not differ (P>0.05) to those of the other studied cultivars.

Table 3. 4. Coefficients of ileal fat (IFD) and dry matter (DMD) digestibility (Obtained with slope ratio method) of ten different UK grown field bean cultivar samples for 21 day-old broiler chickens

Bean	IFD	DMD
Arthur	0.756	0.480[ab]
Buzz	0.591	0.380[ab]
Clipper	0.823	0.441[ab]
Divine	0.816	0.504[ab]
Fuego	0.765	0.522[b]
Fury	0.708	0.482[ab]
Honey	0.824	0.543[b]
Maris Bead	0.712	0.480[ab]
Sultan	0.790	0.345[a]
Wizard	0.712	0.536[b]
Mean	0.750	0.471
CV%	25.6	22.6
SEM (df=63)	0.0678	0.0376
P value	0.353	0.005

Each value represents mean of eight replicate pens of five birds each; CV, coefficient of variation; SEM, standard error of the mean; [a,b]Values within a column with different superscripts differ significantly at $P \leq 0.05$.

The variation in the AME, AMEn, NR, FD and DMR of the field bean cultivar samples were lower than that of the variables determined on digesta (CV=10.9, 11.3, 10.3, 13.8 and 9.8%, respectively) (Table 3. 5). The AME and AMEn ranged from 8.49 and 7.78 MJ/kg DM (Sultan) to 10.98 and 9.96 MJ/kg DM (Divine), respectively.

The AME of the cultivar Sultan did not differ ($P>0.05$) from that of Arthur, Buzz, Clipper and Maris Bead, but was statistically lower ($P<0.05$) than the values of the rest. Nitrogen-corrected apparent metabolisable energy content in field beans followed the same manner as that of AME, except values for Sultan and Honey that were not different ($P>0.05$). The DMR values varied from 0.461 (Sultan) to 0.571 (Divine) amongst cultivars. The DMR of the cultivar Sultan was statistically lower ($P<0.05$) than that of Divine, Fuego, Fury, Honey and Maris Bead but did not differ ($P>0.05$) from the rest. There were no differences ($P>0.05$) in NR and FD between the studied field bean cultivar samples.

Table 3. 5. Energy and nutrient availability (Obtained with slope ratio method) of ten UK grown field bean cultivar samples fed to broiler chickens

Bean	AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR
Arthur	10.07^{abc}	9.19^{abc}	0.596	0.752	0.546^{ab}
Buzz	9.04^{ab}	8.20^{ab}	0.556	0.740	0.491^{ab}
Clipper	10.08^{abc}	9.16^{abc}	0.594	0.903	0.528^{ab}
Divine	10.98^{c}	9.96^{c}	0.624	0.916	0.571^{b}
Fuego	10.67^{bc}	9.78^{bc}	0.606	0.861	0.562^{b}
Fury	10.72^{bc}	9.84^{bc}	0.572	0.868	0.566^{b}
Honey	10.40^{bc}	9.43^{abc}	0.604	0.858	0.550^{b}
Maris Bead	10.29^{abc}	9.35^{abc}	0.558	0.855	0.554^{b}
Sultan	8.49^{a}	7.78^{a}	0.538	0.850	0.461^{a}
Wizard	10.44^{bc}	9.52^{bc}	0.556	0.879	0.547^{ab}
Mean	10.12	9.22	0.580	0.848	0.538
CV%	10.9	11.3	10.3	13.8	9.8
SEM (df=63)	0.390	0.370	0.0211	0.0415	0.0186
P value	<0.001	<0.001	0.091	0.061	0.001

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy, FD, coefficient of total tract fat digestibility; NR, coefficient of total tract nitrogen retention; DMR, coefficient of total tract dry matter retention; Each value represents mean of eight replicate pens of five birds each; Variables were determined from 17 to 21 days of age; CV, coefficient of variation; SEM, standard error of the mean; ^{a,b,c}Values within a column with different superscripts differ significantly at $P \leq 0.05$.

3. 3. 3. Apparent ileal digestibility coefficients of amino acids

Apparent ileal digestibility coefficients (AID) of AAs of the field bean cultivars are summarised in Table 3. 6. The means of AID of the field bean cultivar samples were 0.817 for indispensable AA (CV=5.9%), 0.807 for dispensable AA (CV=5.4%), and 0.811 for total AA (CV=5.4%). The lowest mean digestibility was observed for proline (0.623; CV=12.0%) and the highest for lysine (0.861; CV=4.2%). The mean digestibility of methionine and threonine was 0.821 (CV=12.6%), and 0.810 (CV=7.4%), respectively. The highest variation in digestibility was found for methionine (CV=12.6%) followed by proline (CV=12.0%), tyrosine (CV=9.3%) and histidine (CV=8.6%), although the lowest was for lysine (CV=4.2%) followed by arginine and glutamic acid (CV=4.3%).

There were differences in AA digestibility between the field bean cultivars. Except methionine, the digestibility of all of the studied AAs was significantly different ($P<0.05$) among the bean samples. The lowest AA digestibility was observed for proline in the cultivar Sultan (0.466) followed by Clipper (0.585) and then Arthur (0.593) and the highest digestibility was for lysine in the cultivar Wizard (0.883) followed by Honey (0.882). The lowest and the highest lysine, threonine and cysteine digestibility coefficients were for the cultivars Buzz (0.810) and Wizard (0.883), Sultan (0.703) and Fury (0.853), Sultan (0.680)

and Honey (0.840), respectively. The variation found for digestibility of lysine, threonine and cysteine, was 8.3, 17.6 and 19.0 percentage point, respectively across all cultivars. With the exception of lysine (Buzz had the lowest) the Sultan cultivar sample had the lowest digestibility of all AAs. The cultivar Sultan had the lowest dispensable, indispensable and total AA digestibility coefficients. Wizard had the highest indispensable and total AA, and Honey had the highest dispensable AA digestibility.

Table 3. 6. Apparent ileal digestibility coefficient of amino acids (Obtained with slope ratio method) of ten UK grown field bean cultivar samples for 21-day-old broiler chickens

Amino acid digestibility	Field Bean Cultivar										Mean	CV%	SEM	*P* value
	Arhtur	Buzz	Clipper	Divine	Fuego	Fury	Honey	Maris Bead	Sultan	Wizard			(df=63)	
Indispensable amino acids														
Arginine	0.848^{bc}	0.798^{ab}	0.842^{bc}	0.864^{c}	0.846^{bc}	0.854^{bc}	0.867^{c}	0.876^{c}	0.775^{a}	0.878^{c}	0.845	4.3	0.0127	<0.001
Histidine	0.779^{ab}	0.763^{ab}	0.765^{ab}	0.818^{ab}	0.774^{ab}	0.822^{b}	0.834^{b}	0.839^{b}	0.710^{a}	0.829^{b}	0.793	8.6	0.0241	0.005
Isoleucine	0.825^{b}	0.784^{ab}	0.791^{ab}	0.829^{b}	0.807^{ab}	0.832^{b}	0.844^{b}	0.825^{b}	0.745^{a}	0.835^{b}	0.812	5.5	0.0156	<0.001
Leucine	0.811^{b}	0.773^{ab}	0.769^{ab}	0.822^{b}	0.808^{b}	0.819^{b}	0.830^{b}	0.824^{b}	0.704^{a}	0.825^{b}	0.798	5.8	0.0164	<0.001
Lysine	0.878^{b}	0.810^{a}	0.844^{ab}	0.870^{b}	0.871^{b}	0.864^{ab}	0.882^{b}	0.878^{b}	0.826^{ab}	0.883^{b}	0.861	4.2	0.0126	<0.001
Methionine	0.881	0.756	0.836	0.816	0.825	0.808	0.865	0.802	0.756	0.865	0.821	12.6	0.0367	0.215
Phenylalanine	0.820^{b}	0.767^{ab}	0.760^{ab}	0.806^{b}	0.775^{ab}	0.798^{b}	0.820^{b}	0.800^{b}	0.706^{a}	0.808^{b}	0.786	5.8	0.0160	<0.001
Threonine	0.810^{b}	0.768^{ab}	0.796^{ab}	0.840^{b}	0.810^{b}	0.853^{b}	0.836^{b}	0.849^{b}	0.703^{a}	0.839^{b}	0.810	7.4	0.0213	<0.001
Valine	0.821^{b}	0.778^{ab}	0.798^{ab}	0.829^{b}	0.804^{ab}	0.840^{b}	0.845^{b}	0.834^{b}	0.725^{a}	0.835^{b}	0.811	6.1	0.0174	<0.001
Dispensable amino acids														
Alanine	0.840^{b}	0.780^{ab}	0.803^{ab}	0.841^{b}	0.826^{b}	0.846^{b}	0.854^{b}	0.844^{b}	0.746^{a}	0.849^{b}	0.823	5.9	0.0172	<0.001
Aspartic acid	0.841^{b}	0.805^{ab}	0.829^{ab}	0.857^{b}	0.844^{b}	0.867^{b}	0.858^{b}	0.862^{b}	0.764^{a}	0.860^{b}	0.839	5.0	0.0149	<0.001
Cysteine	0.795^{b}	0.765^{ab}	0.750^{ab}	0.814^{b}	0.812^{b}	0.792^{b}	0.840^{b}	0.801^{b}	0.680^{a}	0.776^{b}	0.782	7.4	0.0205	<0.001
Glutamic acid	0.861^{b}	0.809^{ab}	0.826^{ab}	0.850^{b}	0.833^{ab}	0.848^{b}	0.860^{b}	0.853^{b}	0.776^{a}	0.863^{b}	0.838	4.3	0.0128	<0.001
Glycine	0.803^{b}	0.766^{ab}	0.779^{ab}	0.831^{b}	0.801^{b}	0.840^{b}	0.841^{b}	0.842^{b}	0.708^{a}	0.831^{b}	0.804	6.6	0.0187	<0.001
Proline	0.593^{b}	0.634^{b}	0.585^{ab}	0.674^{b}	0.612^{b}	0.641^{b}	0.691^{b}	0.649^{b}	0.466^{a}	0.685^{b}	0.623	12.0	0.0264	<0.001
Serine	0.812^{b}	0.770^{ab}	0.788^{ab}	0.835^{b}	0.826^{b}	0.831^{b}	0.836^{b}	0.839^{b}	0.712^{a}	0.832^{b}	0.808	5.9	0.0169	<0.001
Tyrosine	0.706^{abc}	0.688^{abc}	0.714^{abc}	0.784^{c}	0.644^{ab}	0.753^{bc}	0.766^{c}	0.752^{bc}	0.642^{a}	0.734^{abc}	0.718	9.3	0.0237	<0.001
Indispensable amino acids	0.832^{b}	0.783^{ab}	0.802^{ab}	0.839^{b}	0.812^{b}	0.837^{b}	0.826^{b}	0.845^{b}	0.745^{a}	0.847^{b}	0.817	5.9	0.0171	0.001
Dispensable amino acids	0.819^{b}	0.778^{ab}	0.785^{ab}	0.829^{b}	0.806^{b}	0.827^{b}	0.836^{b}	0.829^{b}	0.725^{a}	0.833^{b}	0.807	5.4	0.0154	<0.001
Total amino acids	0.825^{b}	0.780^{ab}	0.793^{ab}	0.833^{b}	0.808^{b}	0.832^{b}	0.832^{b}	0.836^{b}	0.734^{a}	0.840^{b}	0.811	5.4	0.0156	<0.001

Each value represents mean of eight replicate pens of five birds each; CV, coefficient of variation; SEM, standard error of the mean; a,b, cValues within a row with different superscripts differ significantly at P≤0.05.

3. 3. 4. Relationship between the chemical composition, physical properties and the availability of nutrients and energy of field beans for broilers

The correlation coefficients were obtained using all the data from the laboratory analysis and broiler experiments (Table 3. 7). The CP content was positively (P<0.05) correlated to determine ME, DMR, DMD and L* FB. The GE and L* FB were correlated positively (P<0.01) to ME, DMR, DMD. The GE and CP of beans were the only laboratory measurements that was significantly correlated with L* FB (P<0.01 and P<0.05, respectively). Condensed tannin contents (P<0.05) and viscosity (P<0.01) had a positive correlation with the FD of field beans. Cotyledon ratio correlated positively to the AA content of the beans (P<0.05). There was a negative relation (P<0.05) for both T-NSP and I-NSPs content with cotyledon ratio in the field beans. A positive correlation (P<0.05) was also observed between total phenols and WHC.

The step-wise regression technique identified the chemical components of the bean samples and the laboratory measures of quality that minimised the residual mean squares for AME and TAAD. The CP was chosen as the first independent variable to L* FB in the following equations in order to provide a clearer interpretation of the results. For AME, the statistically significant explanatory variables were CP content and the amount of ash in the bean cultivar samples (P<0.05). The addition of other explanatory variables did not significantly (P>0.05) reduce the residual mean squares in these dependent variables (Table 3. 9). For TAAD, the statistically significant explanatory variables were CP, ash and T-NSP content of the bean cultivar samples (P<0.05) (Table 3. 10).

Table 3. 7. Correlation coefficients between determined energy and nutrient utilisation, chemical composition and physical properties of UK grown field bean cultivars

	NR	IDAAD	DAAD	TAAD	AME	AMEn	IFD	FD	DMD	DMR	L* FB	GE	CP	TIDAAC	TDAAC	TAAC	Cotyledon	Oil	Starch	T-NSPs	I-NSPs	S-NSPS	DM	Ash	WHC	WEV	Phytate	Tot. phe.	Tannins	CT	TGW
CPR	1.000																														
IDAAD	0.418	1.000																													
DAAD	0.495	0.971	1.000																												
TAAD	0.460	0.991	0.995	1.000																											
AME	0.690	0.877	0.879	0.882	1.000																										
AMEn	0.674	0.867	0.865	0.870	0.998	1.000																									
IFD	0.570	0.025	0.031	0.029	0.287	0.283	1.000																								
FD	0.290	0.255	0.204	0.228	0.512	0.512	0.633	1.000																							
DMD	0.597	0.854	0.902	0.887	0.897	0.891	0.289	0.381	1.000																						
DMR	0.663	0.912	0.913	0.917	0.986	0.985	0.218	0.393	0.894	1.000																					
L* FB	0.618	0.896	0.948	0.931	0.928	0.924	0.037	0.214	0.895	0.947	1.000																				
GE	0.588	0.745	0.783	0.771	0.904	0.910	0.325	0.561	0.911	0.861	0.854	1.000																			
CP	0.304	0.824	0.820	0.828	0.694	0.658	-0.003	0.385	0.674	0.684	0.683	0.551	1.000																		
TIDAAC	0.096	0.370	0.472	0.431	0.201	0.182	-0.442	-0.318	0.203	0.244	0.466	0.242	0.372	1.000																	
TDAAC	0.141	0.361	0.467	0.423	0.254	0.238	-0.381	-0.218	0.221	0.286	0.490	0.310	0.355	0.987	1.000																
TAAC	0.120	0.366	0.471	0.428	0.230	0.212	-0.412	-0.267	0.213	0.267	0.480	0.278	0.364	0.996	0.997	1.000															
Cotyledon	0.151	0.566	0.698	0.646	0.348	0.321	-0.209	-0.226	0.556	0.426	0.582	0.358	0.585	0.683	0.650	0.668	1.000														
Oil	-0.152	-0.307	-0.257	-0.284	-0.248	-0.210	-0.119	-0.423	-0.041	-0.181	-0.157	-0.082	-0.625	-0.270	-0.259	-0.265	-0.023	1.000													
Starch	0.145	-0.048	0.112	0.043	-0.055	-0.044	0.075	-0.420	0.173	0.030	0.155	0.103	-0.298	0.332	0.348	0.342	0.540	0.602	1.000												
T-NSPs	-0.095	-0.359	-0.422	-0.393	-0.192	-0.197	0.137	0.388	-0.349	-0.318	-0.364	-0.238	-0.073	-0.461	-0.462	-0.463	-0.687	-0.468	-0.756	1.000											
I-NSPs	-0.282	-0.204	-0.324	-0.271	-0.135	-0.138	-0.082	0.412	-0.344	-0.245	-0.314	-0.209	0.080	-0.363	-0.364	-0.365	-0.643	-0.546	-0.916	0.918	1.000										
S-NSPS	0.348	-0.465	-0.370	-0.410	-0.195	-0.202	0.506	0.103	-0.152	-0.280	-0.250	-0.156	-0.345	-0.386	-0.387	-0.388	-0.365	-0.026	0.027	0.571	0.197	1.000									
DM	0.100	0.217	0.020	0.100	0.249	0.266	0.140	0.292	-0.024	0.247	0.024	0.126	0.011	-0.216	-0.180	-0.198	-0.492	-0.156	-0.479	0.033	0.245	-0.426	1.000								
Ash	-0.330	-0.454	-0.464	-0.463	-0.412	-0.425	-0.080	0.217	-0.394	-0.525	-0.478	-0.170	-0.113	0.064	0.074	0.069	-0.318	-0.270	-0.391	0.435	0.488	0.065	0.043	1.000							
WHC	-0.063	-0.062	-0.050	-0.054	0.175	0.217	0.383	0.510	0.189	0.145	0.044	0.348	-0.236	-0.383	-0.267	-0.323	-0.137	0.396	0.276	-0.070	-0.105	0.042	-0.037	-0.182	1.000						
WEV	0.069	0.171	0.172	0.174	0.317	0.312	0.472	0.772	0.216	0.263	0.120	0.290	0.376	-0.193	-0.095	-0.142	0.083	-0.367	-0.120	0.152	0.178	0.004	-0.048	-0.058	0.606	1.000					
Phytate	-0.286	0.060	0.079	0.074	-0.055	-0.078	-0.584	-0.050	-0.026	-0.113	0.053	-0.051	0.399	0.244	0.183	0.213	0.105	-0.386	-0.523	0.472	0.571	-0.013	-0.320	0.441	-0.433	-0.147	1.000				
Tot. phe.	-0.261	-0.425	-0.455	-0.444	-0.230	-0.210	0.466	0.574	-0.143	-0.320	-0.460	0.041	-0.321	-0.556	-0.476	-0.516	-0.410	0.229	-0.063	0.210	0.188	0.130	0.092	0.479	0.661	0.451	-0.237	1.000			
Tannins	-0.357	-0.202	-0.297	-0.257	-0.135	-0.111	0.367	0.520	-0.036	-0.186	-0.371	0.078	-0.201	-0.584	-0.528	-0.557	-0.347	0.268	-0.127	0.050	0.137	-0.162	0.300	0.326	0.615	0.386	-0.284	0.919	1.000		
CT	-0.037	-0.251	-0.323	-0.296	0.035	0.060	0.447	0.707	-0.020	-0.074	-0.253	0.243	-0.214	-0.544	-0.442	-0.492	-0.568	0.125	-0.305	0.312	0.345	0.055	0.410	0.490	0.579	0.371	-0.174	0.888	0.834	1.000	
TGW	0.141	0.079	0.212	0.162	-0.045	-0.063	-0.153	-0.446	0.219	-0.045	0.213	0.069	0.062	0.402	0.305	0.352	0.398	0.017	0.321	-0.028	-0.224	0.394	-0.632	0.001	-0.417	-0.514	0.367	-0.436	-0.531	-0.558	1.000

df=8; $P<0.05$ ($0.632 \leq r < 0.765$); $P<0.01$ ($r \geq 0.765$); NR, coefficient of total tract nitrogen retention; IDAAD, coefficient of apparent ileal digestibility of indispensable amino acids; DAAD, coefficient of apparent ileal digestibility of dispensable amino acids; TAAD, coefficient of apparent ileal digestibility of total amino acids; AME; apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; IFD. coefficient of ileal fat digestibility; FD, coefficient of total tract fat digestibility; DMD, coefficient of ileal dry matter digestibility; DMR, coefficient of total tract dry matter retention; L* FB, lightness score of bean flour; GE, gross energy in beans (MJ/kg DM); CP, crude protein in beans (g/kg DM), TIDAAC, total indispensable amino acid content in beans (g/kg CP); TDAAC, total dispensable amino acid content in beans (g/kg CP); TAAC, total amino acid content in beans (g/kg CP); Cotyledon, cotyledon ratio of beans, Oil, oil content in beans (g/kg DM); Starch, total starch content in beans (g/kg DM); T-NSPs, total non-starch polysaccharide contents in beans (g/kg DM); I-NSPs, insoluble non-starch polysaccharide contents in beans (g/kg DM); S-NSPs, soluble non-starch polysaccharide contents in beans (g/kg DM); DM; dry matter content in beans (g/kg); Ash; ash contents in beans (g/kg DM); WHC, water holding capacity of beans (g/kg DM); WEV; water extract viscosity of beans (centipoise); Phytate, phytate content in beans (mg/g DM), Tot. phe., total phenol, as tannic acid equivalents, content in beans (mg/g DM); Tannins, tannins, as tannic acid equivalents, content in beans (mg/g DM); CT, condensed tannins, as leucocyanidin equivalents, content in beans (mg/g DM); TGW, thousand bean grain weight (g).

3. 3. 5. Relationship between amino acid digestibility and the chemical and physical characteristics of the bean samples

Selected results of significant correlations between apparent ileal amino acid digestibility and the analysed physical and chemical characteristics of the field bean samples are presented in Table 3. 8. The results show significant positive correlations between L* FB, GE, CP, cotyledon ratio, arginine and cysteine contents and the apparent ileal digestibility coefficients of the majority of field bean AAs. Methionine digestibility was the only variable that did not correlate (P>0.05) to any of the studied variables. Lysine digestibility coefficients correlated only to L* FB (P<0.05) and GE (P<0.01) of the beans. The digestibility of arginine, threonine, aspartic acid, serine and tyrosine did not correlate (P>0.05) to cotyledon ratio of the beans. Also there was no significant correlation (P>0.05) between tyrosine digestibility and GE level in the beans.

Table 3. 8. Significant correlation coefficients between coefficients of apparent ileal amino acid digestibility and chemical and physical characteristics of field beans

Amino acid digestibility	L* FB[1]	GE (MJ/kg DM)	CP (g/kg DM)	Arginine (g/kg CP)	Cysteine (g/kg CP)	Cotyledon ratio
Indispensable amino acids						
Arginine	0.853	0.785	0.855		0.639	
Histidine	0.840	0.712	0.900	0.844	0.752	0.744
Isoleucine	0.938	0.790	0.779	0.727	0.811	0.719
Leucine	0.960	0.780	0.794	0.744	0.802	0.716
Lysine	0.755	0.790				
Methionine						
Phenylalanine	0.895	0.666	0.734	0.709	0.774	0.746
Threonine	0.936	0.777	0.843	0.729	0.748	
Valine	0.939	0.759	0.822	0.709	0.756	0.671
Dispensable amino acids						
Alanine	0.938	0.816	0.762	0.661	0.767	0.650
Aspartic acid	0.950	0.811	0.815	0.684	0.746	
Cysteine	0.915	0.720	0.669		0.801	0.749
Glutamic acid	0.896	0.733	0.767	0.634	0.705	0.643
Glycine	0.931	0.782	0.846	0.776	0.790	0.691
Proline	0.853	0.686	0.880	0.831	0.784	0.750
Serine	0.958	0.819	0.807	0.687	0.768	
Tyrosine	0.651		0.846	0.776	0.710	

[1]Lightness score of bean flour; df=8; P<0.05 ($0.632 \leq r^2 < 0.765$); P<0.01 ($r^2 \geq 0.765$).

Table 3. 9. Relationship between apparent metabolisable energy content of the studied field bean cultivars and their chemical composition

Explanatory variables Response variable	Constant	CP	Ash	r^{2a}	SE[b]	*P* value
AME	1.75 (SE=3.070)	0.0296 (SE=0.01090)		0.417	0.593	0.026
AME	5.79 (SE=4.090)	0.0280 (SE=0.01030)	-0.0997 (SE=0.07130)	0.480	0.561	0.042

[a]Corrected r^2; [b] Standard error of observations.

Table 3. 10. Relationship between amino acid utilisation and chemical composition of the studied field bean cultivars

Explanatory variables Response variable	Constant	CP	Ash	T-NSPs	r^{2a}	SE[b]	*P* value
Total AA availability	0.378 (SE= 0.1040)	0.001533 (SE=0.0003680)			0.646	0.0201	0.003
Total AA availability	0.572 (SE=0.1170)	0.001455 (SE=0.0002960)	-0.00479 (SE= 0.002040)		0.773	0.0161	0.002
Total AA availability	0.578 (SE=0.1130)	0.001445 (SE=0.0002850)	-0.00361 (SE= 0.002180)	-0.000251 (SE=0.0001990)	0.790	0.0155	0.006

[a]Corrected r^2; [b] Standard error of observations.

3. 4. Discussion

The purpose of this experiment was to determine the range of variation in energy and nutrient availability of different UK grown field bean cultivar samples when added at 200 g/kg in diets fed to broilers. A further aim was to determine how their energy and nutrient utilisation relates to their chemical and physical characteristics.

3. 4. 1. Broiler performance

The overall final body weight of the birds in all dietary treatments was in the expected range for Ross 308 broilers fed on mash diets (Pirgozliev *et al.,* 2015c), as FCR was similar to breeder's recommendations (Aviagen Ltd, Edinburgh). The differences in the birds FI and WG were not statistically significant in this study, although there were some differences in FCR. This is in agreement with previous reports (Metayer *et al.,* 2003; Nalle *et al.,* 2010a) when a similar amount of dietary field beans were fed to broilers for the same feeding period. The lack of response of growth performance variables to dietary bean cultivars reported by Usayran *et al.* (2014) may be due to the relatively short feeding period (7 days only) and the use of tannin-free bean cultivars.

3. 4. 2. Energy availability of field beans for broilers

There was a difference of 2.49 and 2.18 MJ/kg DM in AME and AMEn among the studied field bean cultivars. The determined AME of the beans, 10.12 MJ/kg DM on average (CV%=10.98), had a similar range to that reported for New Zealand (Nalle, 2009) field bean (9.15-11.97; 10.64 MJ/kg DM) and pea (9.82-10.78; 10.33 MJ/kg DM) cultivars based on five week-old male Ross 308 broilers. The overall determined AMEn value of the field beans was 9.22 MJ/kg DM (CV%=9.96), which is similar to the values reported for field bean (8.25-10.6; 9.54 MJ/kg DM) and pea (9.11-10.16; 9.74 MJ/kg DM) cultivars for broilers (Nalle, 2009). The mean AME value in the field bean samples was quite high and similar to that reported for SBM (10.00 MJ/kg DM). However, the variability was lower compared to that of 55 different SBM (7.38-11.93 MJ/kg DM; 4.45 MJ/kg DM difference) samples reported with 34 days old male Ross 308 broilers by Ravindran *et al.* (2014).

However, Vilariño *et al.* (2009) found higher AMEn values in reconstituted (mixture of cotyledons and hulls from different samples) field beans when fed to broilers at a similar age. The AMEn values (11.22-13.45 MJ/kg DM) of beans determined with cockerels (fed in both mash and pelleted form) were higher compared to the values obtained with growing broilers in this study (Grosjean *et al.,* 1995, 2000; Brévault *et al.,* 2001, 2003; Metayer *et al.,* 2003; Métayer and Vilariño, 2006). These dissimilarities in AMEn results were possibly due to the better developed gastrointestinal tract of the mature birds and pelleting. The use of different field bean cultivars, grinding degree, pelleting, differences in broiler breed and conditions under which birds were reared may also have contributed to the observed variation in metabolisable energy contents between this study and those reported in the literature.

3. 4. 3. Nutrient availability of field beans for broilers

The difference for NR was up to 13.78% among the investigated field bean cultivars in this study. The overall mean of NR of the examined field bean cultivars in this experiment was 0.580 (CV%=10.3). The NR values determined in these field bean samples were low and did not fall within the ranges 0.669-0.826 (Lacassagne *et al.,* 1988), 0.694-0.842 (Lacassagne *et al.,* 1991), 0.764-0.800 (Brufau *et al.,* 1998), 0.826-0.906 (Metayer *et al.,* 2003) and 0.741-0.879 (Vilariño *et al.,* 2009) reported for different field bean cultivars for broilers. Whereas the observed range of difference in NR, due to cultivar variation in this study, was comparable to the 19.0% (Lacassagne *et al.,* 1988), 17.6% (Lacassagne *et al.,*1991), 8.8% (Metayer *et al.,* 2003) 15.7% (Vilariño *et al.,* 2009) reported among individual field bean cultivars for young broilers. These dissimilarities in field bean protein availability probably reflect cultivar influences and differences in the measuring methods (Brufau *et al.,* 1998). Also, the level of raw field beans in the diets and the age of the experimental birds may be other reasons for different determined NR in these studies.

In this study, cultivar had significant impacts on apparent ileal digestibility coefficients (AID) of AAs in field beans. The means of AID of indispensable, dispensable and total AAs were 0.817, 0.807 and 0.811, respectively. The averages of AID of arginine, lysine, methionine, alanine, aspartic acid and glutamic acid were high (0.845, 0.861, 0.821, 0.823, 0.839 and 0.838, respectively) but those of proline and tyrosine were low. The low digestibility values for proline (0.623) and tyrosine (0.718) may be due to low concentrations of these AAs in the studied bean samples, which ranged from 11.7 to 13.6 and 4.4 to 8.5 g/kg DM, respectively. Location of concentration of tyrosine in the field beans may be an additional factor contributing to the low digestibility of this AA. According to Marquardt *et al.* (1975), the concentration of tyrosine is 60 g/kg CP in the hull and is 34 g/kg CP in cotyledons of field beans. The highest variation in AID was for methionine, followed by proline, tyrosine and histidine with CV% of 12.6, 12.0, 9.3 and 8.6, respectively. Apparent ileal amino acid digestibility coefficient values of these field beans were within the range observed in four New Zealand field beans measured with 35 day old male Ross 308 broilers (Nalle *et al.,* 2010a, b). The obtained AID values of AAs from most of the cultivars in this trial were also similar to AID of AAs of two field bean cultivars reported with 21 day old male Ross 308 broilers by Woyengo and Nyachoti (2012).

Coefficients of standardised ileal digestibility of amino acids measured for eight UK field beans with 28 day old male Ross 308 broiler chickens by O'Neill *et al.* (2012), and a faba bean cultivar measured with 30 day old broiler chickens by Szczurek (2009) were similar to AID of AAs found for the majority of bean cultivars evaluated in this study.

The AID of AAs of the current field bean cultivars were in the range of standardised ileal amino acid digestibility coefficents reported for several UK (O'Neill *et al.,* 2012) and New Zealand pea cultivars (Nalle, 2009), and for 55 SBM samples collected from different origins for broilers (Ravindran *et al.,* 2014). The AID of AAs of the used field bean cultivars in this feeding experiment were similar to those of other high protein sources such as chickpeas, cottonseed meal and lupin seeds, and close to those of maize gluten meal, sunflower meal and canola meal reported by Ravindran *et al.* (2005) with 6-week-old broilers.

The examined field bean cultivars did not differ from each other in terms of their IFD and FD. This observation is in agreement with that of Marquardt and Ward (1979), who did not find any difference in FD with young broiler chicks that consumed different field bean cultivars. The overall determined IFD and FD for the bean samples was 0.750 (CV%=25.6) and 0.848 (CV%=13.8), respectively, and the FD of these field bean cultivars was in agreement with values (0.76-0.79) documented by Marquardt and Ward (1979) for different field bean cultivars for broilers. The lack of significant differences in IFD and FD in this study may have been due to the low concentrations of fat and the small range (9.2-12.9 g/kg DM) between the studied field bean samples. In addition, there is a lack of an

adverse impact of anti-nutrients, such as tannins in field beans on lipase enzyme activity and fat utilisation.

The DMD and DMR were statistically different amongst the field bean samples and their averages were 0.471 (CV%=22.6) and 0.538 (CV%=9.8), and their variations were 36.5% and 19.3%, correspondingly. The DMR values found in the studied field bean cultivars in this experiment were close to those (0.423-0.479) documented by Marquardt and Ward (1979) in field beans for young broilers. Higher values of FD and DMR than that of IFD and DMD may have been due to microbial fermentation in the caeca of the birds, as well as existing uric acid in excreta output.

Generally, the differences in the values of energy and nutrient availability between various field bean cultivar samples used in the same or different feeding experiments is related to the variation between the cultivars, in terms of their chemical composition and physical caracteristics. These differences are also a reflection of growing location, growing season, harvest year, bird age and the method of determination of energy and nutrient utilisation (Brufau *et al.,* 1998; Choct and Hughes, 1999; Duc *et al.,* 1999).

3. 4. 4. Relationship between the chemical composition, physical properties and the availability of nutrients and energy of field beans for broilers

The correlation analysis indicated that the concentration of CP in field beans was the major variable that strongly correlated with the utilisation of AAs, AME and AMEn. Since AAs are the basic units of CP, it was expected that AA availability would have a high positive correlation with CP content of the field beans.

Although starch, oil and protein are the main sources of energy in diets, surprisingly in this study starch and oil did not relate to ME contents in field beans. Whereas significant correlation coefficients between AME and AMEn contents and CP content in field beans were observed. Thereby, suggesting that CP was the main determinant nutrient as a source of energy in the studied field bean cultivars.

Availability of AAs, AME and AMEn were strongly positively correlated to L* BF in the current study. The positive correlation of nutrient and energy utilisation of field beans with lightness score (L*) in this study were in agreement with those documented for peas with adult cockerels by Igbasan *et al.* (1997). These researchers found higher true AA availability, AME and AMEn contents in light (both yellow and green) pea cultivars than those in dark (brown) ones with adult leghorn cockerels.

The high positive relationship between L* FB and AA digestibility in the current trial was due to a strong correlation of CP concentration in field beans with L* FB (r=0.683). Since cotyledon ratio had a strong positive relationship with CP, total AA (r=0.668), total indispensable (r=0.683) and dispensable (r=0.650) and the majority of individual AA contents in the field beans, the high positive correlation between cotyledon ratio and AA digestibility was not surprising in this study.

There is a general agreement that pale legume seeds have higher nutritive value than dark seeded cultivars. Overall, light hull field beans contain slightly lower levels of one or more anti-nutrients, such as phenols and tannins (Lindgren, 1975; Helsper *et al.,* 1993; Duc *et al.,* 1995; Oomah *et al.,* 2011), phytate (Rubio *et al.,* 1992; Oomah *et al.,* 2011), fibres, lignin (Helsper *et al.,* 1993; Igbasan *et al.,* 1997; Duc *et al.,* 1999), but higher phytase (Oomah *et al.,* 2011) and CP than dark hull colour cultivars. Moreover, Duc *et al.* (1995) observed that tannin activity in field beans is strongly associated with the darkness of their hulls. As deleterious impacts of NSPs (Lacassagne *et al.,* 1991; Longstaff and McNab, 1991a, b; Nalle, 2009), tannins (Ortiz *et al.,* 1993; Brufau *et al.,* 1998; Vilariño *et al.,* 2009; Woyengo and Nyachoti, 2012) and phytate (Cowieson *et al.,* 2004) on energy, CP and AA utilisation of field beans by broilers have been noted previously.

No statistical significant relationship between AA availability of the field beans and their tannin and phytate contents was detected in this experiment. Lack of significant correlation between tannins and ileal AA digestibility of field beans in this study agreed with that previously reported for eight UK field beans by O'Neill *et al.* (2012). These researchers did not detect any significant correlation between standardised ileal digestibility of AAs and tannin contents of six T- and two T+ UK field bean cultivars when fed at 750 g/kg diet to 28 day male Ross 308.

The lack of statistical significant correlations between colour score and phenols and tannin contents could be due to oxidation of phenols and tannins, thus altering low molecular weight phenols and tannins to polymerised high molecular weight compounds. It has been stated that an increase in darkness degree occurs due to oxidation of phenolic compounds, such as condensed tannin contents in field beans (Marquardt *et al.,* 1978; Black and Brouwer, 1998; Nasar-Abbas *et al.,* 2008, 2009). This oxidation reaction increases the polymerisation of exciting low molecular weight phenolic compounds to high molecular weight resultants (that are not extractable during lab analysis), which leads to an increase in the darkness score of beans (Nozzolillo and Bezada, 1984). Thereby, low concentrations of phenols and tannins are detected during laboratorial analysis (Marquardt *et al.,* 1978) in dark seed-coated field beans. According to Beninger *et al.* (2005), a higher degree of darkness in beans indicates initial high levels of condensed tannins in before oxidation reactions and polymerisation, which sometimes can occur on farm before harvesting or during storage.

In contrast to the findings of this study, Brufau *et al.* (1998) and Vilariño *et al.* (2009) found a negative relation between tannin level and AME and AMEn contents in field beans. These authors compared only a few high tannin-containing and T- field bean cultivars when fed to young chicks, while all field bean cultivars used in this study contained some tannins that may be the reason for the lack of similarities with these observations.

The step-wise multiple regression analysis showed that the amount of CP, ash and T-NSPs were the major compositional variables of field beans that significantly related to AA

digestibility, when the CP and ash contents were the best compositional variables that had a significant correlation with AME content in the field beans for broilers.

The content of ash was the second variable that contributed to the explanation of the variation in the AID of AAs and AME values of the bean samples for broiler chickens. The range of ash contents for the bean samples was 32.0 to 39.4 g/kg DM. Although significant regression coefficients were obtained, they indicated that a 1 g/kg DM increase in ash gave only a relatively small decrease (which may be not commercially significant) in AID of AAs and AME. The small differences that ash content appeared to explain in AID of AAs and AME may not be due to direct effects of ash on the nutritive value of the field beans but could be an indication of some seed chemical composition variables that negatively affect AID of AAs and AME. Among the studied field beans ash concentration had a positive correlation with the contents of NSP, condensed tannins, phytate, but had a negative correlation with L* FB and cotyledon ratio. Rubio *et al.* (1992) also noticed the relation of ash concentration with these mentioned variables in field beans.

The ash content of different field bean samples can vary due to cultivar (Makkar *et al.,* 1997), growing season (Eden, 1968), growing location (Harmankaya *et al.,* 2010; Ravindran *et al.,* 2014), soil contamination and crop pathogen (Zelalem and Chandravanshi, 2014). The differences in ash content observed in the present experimental bean samples may have resulted from differences in the crop growth and grain development characteristics for the different cultivar samples under the specific crop husbandry conditions and pathogen infestations. However, with the removing these factors the variation in ash content may be eliminated.

The negative correlation of field bean NSPs with AA digestibility observed in this report is in line with that of previous reports (Longstaff and McNab, 1991a, b; Nalle, 2009). Non-starch polysaccharide composition of field beans is complex (Longstaff and McNab, 1991a; Perez-Maldonado *et al.,* 1999). Differences in NSP and NSP constitutional sugars between different cultivars for field beans have been detected (Rubio *et al.,* 1992), and thus their effects on AA digestibility remain speculative. Nevertheless, they might have contributed to different effects of NSPs in the gut, even though their concentration was similar among genotypes. The negative influence of field bean NSPs on AA digestibility could be a consequence of their role in adsorption of protease enzymes in the gut of broilers or their role as a physical barrier that limits the accessibility of proteolytic enzymes to the capsulated CP due to cell wall materials (Longstaff and McNab, 1991a). Padilla *et al.* (2005) suggested that NSPs may affect the ileal flow of AAs and peptides in birds, which could be the outcome of raised secretion of endogenous proteins into the intestine of birds. Non-starch polysaccharides could also interact with the intestinal wall and cause changes to the activity of peptide hormones, which control intestine roles. These changes comprise of invigoration of secretion of endogenous protein or the raised nitrogen flow,

which could reflect the raised secretion of mucins, as an allergic/antigen response (Angkanaporn *et al.,* 1994).

Coefficient of total tract fat digestibility of the examined field bean cultivars positively correlated to their condensed tannin contents (r=0.707) and water extract viscosity (0.772). Improvements in FD in field bean-containing diets for broilers with condensed tannins have been widely documented in literature (Marquardt and Ward, 1979; Longstaff and McNab, 1991a, b; Longstaff *et al.,* 1993). The enhancement in field bean fat digestibility with condensed tannins is due to increases in the activity of lipase in the jejunum and ileum of birds with condensed tannins (Longstaff and McNab, 1991b). The positive relationship of FD and water extract viscosity could possibly be the consequence of increasing transit time of digesta in the intestine, thus allowing more fat digestibility and absoroption.

3. 5. Conclusion

The results of this experiment indicated that these ten field bean cultivars had different energy and nutrient availability for male Ross 308.

There is an indication from these samples that the feeding quality of different field bean cultivars can be predicted by their CP contents, cotyledon ratio and L* FB. The information may be of practical importance to plant breeders who may be able to incorporate improved CP, cotyledon proportion and L* FB traits in their development of new field bean cultivars. The experimental diets were all fed in mash form and without exogenous enzymes in the present experiment. Future studies are necessary to confirm whether the relationship between these explanatory variables and dependant variables remain when enzyme supplements or pelleted feeds are given. Future work should also examine whether these characteristics could be used to discriminate between field bean samples from different growing sites that are received at commercial feed mills.

There is no available information showing the influence of commercially available exogenous enzymes on the feeding quality of field beans that differ in their compositional profiles for broilers. Therefore, further research is needed to investigate whether the addition of commercially available enzymes (phytase, xylanase and protease) can enhance energy and nutrient utilisation of these field bean cultivars and reduce the variations in nutritive value between cultivars for broiler chickens. For this purpose, it is necessary to investigate the effects of enzyme supplementation, either individually, in pairs and in combination to low, medium and high NSPs, phytate and protein containing field bean cultivars.

CHAPTER 4: NUTRITIONAL VALUE OF DIETS BASED ON THREE DIFFERENT FIELD BEAN CULTIVARS WITH AND WITHOUT PHYTASE, XYLANASE AND PROTEASE ENZYME SUPPLEMENTATION FOR BROILERS

4. 1. Introduction

It is known that field bean NSPs (Longstaff and McNab, 1991a, b) and dietary phytate (Cowieson *et al.,* 2004) reduce the bioavailability of nutrients for growing broilers. Amongst the ten various field bean cultivars studied in the previous experiment, Divine had the greatest, Clipper had medium and Buzz had very low AME and nutrient availability. Amongst the studied field bean cultivars, Divine had high protein and low phytate contents, while Buzz contained low protein and the highest phytate. The concentrations of protein and phytate were medium in Clipper field beans. The concentration of NSPs was the highest in Clipper, medium in Buzz and low in Divine field beans.

Based on the chemical composition, performance, energy and nutrient availability information from the previous studies, an experimental design was proposed to use the three field bean cultivar samples referred to above. The general aim of this experiment was to investigate the utilisation of dietary energy and nutrients, gastrointestinal tract components and performance of 7 to 21 day-old broiler chickens, when fed these three field bean cultivar samples as a part of complete diets. These will be fed with and without supplementation of phytase, xylanase and protease either singularly, in pairs and as a mixture of them all together. This is to examine if there is any significant interaction between the treatment factors. In this study, the inclusion rate of 300 g/kg field beans was used to provide more substrates to the enzymes and to provide a wider range of variability in phytate, NSPs and protein content, between the experimental diets. Additionally, this gives the ability to test whether the dietary inclusion rate of field beans can be increased by including exogenous enzymes.

4. 2. Materials and methods

4. 2. 1. Diet formulation

A wheat-soybean based balancer feed was formulated that had major ingredients of 578 g/kg wheat and 182 g/kg full fat SBM, and contained 197 g/kg CP and 14.97 MJ/kg ME. Three diets containing 300 g/kg of one of the three freshly hammer-milled (passing a 4 mm screen) experimental field bean samples (Buzz, Clipper and Divine) were then prepared after mixing with 700 g/kg of the balancer feed (Table 4. 1). Each diet was then split into eight equal batches, seven of which were supplemented with commercial phytase (Phyzyme® XP), xylanase (Danisco Xylanase) or protease (Axtra PRO) both

singularly and in combination. One sample from each of the eight batches was not supplemented, resulting in 24 dietary treatments in total (Table 4. 2).

Table 4. 1. Ingredient composition (g/kg, as-fed) of the experimental broiler chicken field beans diet formulations

Ingredient	Buzz	Clipper	Divine
Wheat	404.2	404.2	404.2
Buzz bean	300	-	-
Clipper bean	-	300	-
Divine bean	-	-	300
SBM (CP=48%)	27.0	27.0	27.0
Full fat soybean meal	127.5	127.5	127.5
Maize gluten meal	35.0	35.0	35.0
Soy oil	65.0	65.0	65.0
Lysine	2.3	2.3	2.3
Methionine	5.8	5.8	5.8
Threonine	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0
Limestone	14.0	14.0	14.0
Sodium chloride	2.8	2.8	2.8
Vitamin/mineral premix*	4.0	4.0	4.0
Total	1000	1000	1000
Analysed values (as-fed)			
GE (MJ/kg)	17.60	17.67	17.69
CP	189	194	199
Fat	95	95	95
Calculated composition			
ME (MJ/kg)	12.77	13.06	13.33
Protein (g/kg)	221	224	229
Lysine (g/kg)	12.8	12.1	13.0
Methionine + cysteine (g/kg)	8.5	8.6	8.5
Calcium (g/kg)	8.2	8.3	8.2
Total phosphorus (g/kg)	6.4	6.3	6.1
available phosphorus (g/kg)	4.0	3.9	3.8
Sodium (g/kg)	1.5	1.5	1.5

This balancer was fed as part of complete diet comprising 300 g/kg of each experimental field bean sample and 700 g/kg of the balancer. Each experimental diet met or exceeded the diet specification for this strain of broiler chicken (Aviagen Ltd., Edinburgh, UK).
* Vitamin and mineral premix provided per kg diet: 50 mg nicotinic acid, 34 mg α-tocopherol, 15 mg pantothenic acid, 7 mg riboflavin, 5 mg pyridoxine, 3.6 mg retinol, 3 mg menadione, 2 mg thiamine, 1 mg folic acid, 200 *μ*g biotin, 125 *μ*g cholecalciferol, 15 *μ*g cobalamin, 100 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 1 mg iodine, 0.5 mg cobalt, 0.5 mg molybdenum and 0.2 mg selenium.

All enzymes were provided by Danisco Animal Nutrition (DuPont Industrial Biosciences, Marlborough, UK). Xylanase (EC 3.2.1.8) is a fungal endo-1,4-ß-xylanase biosynthesised from *Trichoderma reesei*. Phyzyme® XP (EC 3.1.3.26) is a bacterial 6-phytase, produced

from a species of *Escherichia coli* and is expressed in a *Saccharomyces pombe*. Axtra PRO (EC 3.4.21.62) is a subtilisin protease, expressed in *Bacillus subtilis*. Enzymes were added on top of the diets, in powder form, providing an activity of 1000, 2000 and 4000 units/kg diet (U/kg) for phytase, xylanase and protease, respectively. No adjustments were made for differences in dry matter between the field bean samples because only a small range of differences were observed and deemed insignificant. All diets were supplied with 5 g/kg of titanium dioxide (TiO_2) as an indigestible marker to determine marker based digestibility of DM and nitrogen in the ileum. All diets were fed as mash.

Table 4. 2. Experimental dietary treatments

Diet	Bean	Phytase	Xylanase	Protease
1	Divine	-	-	-
2	Clipper	-	-	-
3	Buzz	-	-	-
4	Divine	+	-	-
5	Clipper	+	-	-
6	Buzz	+	-	-
7	Divine	-	+	-
8	Clipper	-	+	-
9	Buzz	-	+	-
10	Divine	-	-	+
11	Clipper	-	-	+
12	Buzz	-	-	+
13	Divine	+	+	-
14	Clipper	+	+	-
15	Buzz	+	+	-
16	Divine	+	-	+
17	Clipper	+	-	+
18	Buzz	+	-	+
19	Divine	-	+	+
20	Clipper	-	+	+
21	Buzz	-	+	+
22	Divine	+	+	+
23	Clipper	+	+	+
24	Buzz	+	+	+

4. 2. 2. Bird husbandry, experimental design and sample collection

The experiment was approved by the Research Ethics Committee at Harper Adams University.

Male Ross 308 broiler chicks were obtained from a commercial hatchery at one-day old and were placed in a single floor pen and fed on a proprietary broiler starter feed until 6 days of age. On the first day of the experimental period (at 7 days of age), the chicks were individually weighed and randomly placed in one of the experimental pens. Two birds were placed in each pen (0.16 m^2 solid floor area) within a controlled environment. Each of the 24 experimental diets was allocated at random to 9 pens within 9 random blocks from 7 to 21 days of age, resulting in 216 pens. The temperature was kept at 30^oC until the chickens were 7 days of age and was gradually reduced to 23^oC at the end of the 14 day experimental feeding period. A standard lighting programme for broilers was used, decreasing the light: dark ratio from 23 hours: 1 hour from day old to 18 hours: 6 hours at 7 days of age, which was maintained until the end of the study. Access to feed and water was *ad libitum* throughout the experimental period.

During the last four days of the experiment (from 18-21 days of age), the solid floor of each pen was replaced with a wire mesh and all excreta were collected and immediately dried at 60^oC. The dried excreta were left at room temperature for three nights and weighed, before milling, to pass through a 0.8 mm mesh. Feed intakes were also measured for the same period. At the end of the study (at 21 days of age), the two birds in each pen were killed by cervical dislocation, weighed, ileal digesta were collected, on a per pen basis, pooled and freeze-dried, and then milled to pass through a 0.8 mm sieve.

The empty weights of the gastrointestinal tract (GIT) segments including, proventriculus and gizzard (PG), pancreas, jejunum and ileum of the heavier bird in each pen were taken according to the procedure described by Amerah and Ravindran (2008). The heavier bird in each pen was selected for measuring GIT segments, as it was considered to be more responsive to the dietary treatments. The weight of each of the segments was expressed as relative to live BW.

The GE, DM, nitrogen and fat of each dried excreta sample and the experimental diets, as well as nitrogen in the digesta were determined, as explained previously (see Section 2. 2. 2. 1). Titanium concentration in the balancer diet and digesta was determined using the method described by Short *et al.* (1996). Apparent metabolisable energy (AME) and nitrogen-corrected AME (AMEn) contents of the diets were calculated according to the method of Hill and Anderson (1958). The coefficients of total tract fat digestibility (FD), dry matter (DMR) and nitrogen (NR) retention were determined as the difference between intake and excretion (retention) of each nutrient, divided by the respective intake as mentioned before (see Section 3. 2. 3). The coefficents of ileal nitrogen (ND) and dry matter (DMD) digestibility of diets were also determined using TiO_2 concentration as an indigestible marker, as mentioned previously (see Section 3. 2. 3). The means of daily

feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) were also measured over 14 days (from 7 to 21 days of age) of the experiment.

4. 2. 3. Statistical analyses

Statistical analyses were performed using the Genstat statistical software package (Genstat 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The data was compared statistically by analysis of variance (ANOVA) using a 3 x 2 x 2 x 2 factorial arrangement of treatments. The main effects were the three field bean cultivars and enzyme supplementation (with and withought phytase, xylanase and protease single, in pairs or in combination), giving a total of twenty-four dietary treatments (Table 4. 2). Interactions between the experimental treatment factors were also tested. Means were separated using a Tukey's test. In all instances, differences were reported as significant at $P \leq 0.05$. Tendencies towards significance were also reported at $P \leq 0.10$.

4. 3. Results

4. 3. 1. Effect of the experimental diets on growth performance variables

Results on growth performance are presented in Table 4. 3, and data on observed interactions is shown in Tables 4. 3 A, 4. 3 B, 4. 3 C and 4. 3 D. However, field bean cultivar did not affect FI, but feeding Divine-based diets resulted in higher ($P<0.05$) WG and lower FCR ($P<0.001$) than those observed with Buzz and Clipper-based diets. Phytase supplementation increased FI ($P=001$), WG ($P<0.01$) and reduced FCR ($P=005$) by 4.4, 6.5 and 1.98%, respectively. Although xylanase inclusion reduced ($P<0.05$) FI by 2.6%, but did not cause any change ($P>0.05$) in WG and FCR. Supplementation of protease did not lead to any alteration ($P>0.05$) in any of the growth performance variables.

However, there was no interaction for FCR between the effect of the field bean variety and exogenous enzymes (Tables 4. 3), but there were phytase by xylanase and phytase by xylanase by protease interactions ($P<0.05$) for FI (Tables 4. 3 A, 4. 3 B) and WG (Tables 4. 3 C, 4. 3 D). While phytase alone improved ($P<0.05$) each variable, and protease also tended to to improve ($P>0.05$) FI and WG, xylanase tended to ($P>0.05$) reduce both variables. A combination of the three enzymes simultaneously ($P<0.05$) improved FI by approximately 5.5% (Table 4. 3 B), and WG by approximately 8.5% (Table 4. 3 D), which were not significantly higher than improvements with supplementation of phytase alone.

Table 4. 3. Effect of experimental diets on growth performance of broilers

Treatment factor	FI (DM g/b/d)	WG (g/b/d)	FCR (g:g)
Beans (B)			
Buzz	52.7	40.3[b]	1.308[b]
Clipper	53.2	40.6[b]	1.315[b]
Divine	53.2	42.0[a]	1.269[a]
SEM	0.60	0.49	0.0077
Enzyme			
Phytase (Phyt)			
No	51.9	39.7	1.310
Yes	54.2	42.3	1.284
Xylanase (Xyl)			
No	53.7	41.3	1.304
Yes	52.3	40.6	1.291
Protease (Prot)			
No	52.8	40.9	1.294
Yes	53.2	41.0	1.300
SEM (df=181)	0.49	0.40	0.0063
P values			
Beans	0.784	0.040	<0.001
Phytase	0.001	<0.001	0.005
Xylanase	0.046	0.257	0.149
Protease	0.606	0.946	0.483
Interactions			
B x Phyt	0.803	0.636	0.620
B x Xyl	0.983	0.755	0.289
B x Prot	0.888	0.868	0.233
Phyt x Xyl	0.014*	0.006***	0.400
Phyt x Prot	0.406	0.827	0.240
Xyl x Prot	0.578	0.782	0.616
Phyt x Xyl x Prot	0.025**	0.017****	0.706
B x Phyt x Xyl	0.235	0.235	0.921
B x Phyt x Prot	0.442	0.406	0.880
B x Xyl x Prot	0.363	0.427	0.422
B x Phyt x Xyl x Prot	0.135	0.091	0.680

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; Each mean represents values from nine replicate pens of two birds each; Bird performance was determined from 7 to 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$. [a,b]Values within a column with different superscripts differ significantly.

***Table 4. 3 A.** Interaction of phytase and xylanase on FI of broiler chickens (SEM = 0.70) (see Table 4. 3)

Phytase	Xylanase	No	Yes
No		53.4	50.3
Yes		54.0	54.4

****Table 4. 3 B.** Interactions of phytase, xylanase and protease on FI of broiler chickens (SEM = 0.99) (see Table 4. 3)

	Xylanase	No	No	Yes	No
Phytase	Protease	No	Yes	No	Yes
No		52.9	53.9	51.0	49.6
Yes		54.5	53.5	52.9	55.8

*****Table 4. 3 C.** Interaction of phytase and xylanase on WG of broiler chickens (SEM = 0.56) (see Table 4. 3)

Phytase	Xylanase	No	Yes
No		40.8	38.6
Yes		41.8	42.7

******Table 4. 3 D.** Interactions of phytase, xylanase and protease on WG of broiler chickens (SEM = 0.79) (see Table 4. 3)

	Xylanase	No	No	Yes	No
Phytase	Protease	No	Yes	No	Yes
No		40.2	41.3	39.2	37.9
Yes		42.5	41.1	41.9	43.6

4. 3. 2. Energy and nutrient availability of the experimental diets

Results on dietary energy and nutrient utilisation are summarised in Table 4. 4, and interactions in tables 4. 4 A, 4. 4 B and 4. 4 C. Diets based on Divine bean cultivar had higher (P<0.05) AME, NR, FD and DMD, and (P<0.001) DMR coefficient contents than those based on Buzz or Clipper, and the values were similar (P>0.05) between the two latter cultivar based diets.

However exogenous phytase supplementation did not lead to any change (P>0.05) in AME, DMR and NR, but increased ND (P<0.05) by 1.4%, and decreased (P<0.001) FD by 4.3%, and tended to (P=0.07) reduce AMEn.

Table 4. 4. Energy and nutrient availability of the experimental diets for broilers

Treatment factor	AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR	ND	DMD
Beans (B)							
Buzz	14.41[b]	13.60	0.673[b]	0.755[b]	0.684[b]	0.719	0.627[b]
Clipper	14.41[b]	13.58	0.674[b]	0.768[b]	0.683[b]	0.718	0.622[b]
Divine	14.56[a]	13.70	0.685[a]	0.791[a]	0.693[a]	0.719	0.637[a]
SEM	0.048	0.046	0.0029	0.0074	0.0020	0.0036	0.0033
Enzyme							
Phytase (Phyt)							
No	14.50	13.68	0.675	0.788	0.687	0.714	0.626
Yes	14.41	13.58	0.680	0.754	0.686	0.724	0.632
Xylanase (Xyl)							
No	14.37	13.54	0.679	0.765	0.683	0.719	0.625
Yes	14.55	13.72	0.676	0.777	0.690	0.719	0.632
Protease (Prot)							
No	14.46	13.63	0.677	0.775	0.686	0.718	0.629
Yes	14.45	13.62	0.678	0.768	0.687	0.720	0.628
SEM (df=181)	0.039	0.038	0.0024	0.0061	0.0016	0.0030	0.0027
P values							
Beans	0.042	0.158	0.006	0.003	<0.001	0.920	0.008
Phytase	0.101	0.070	0.130	<0.001	0.517	0.021	0.092
Xylanase	0.002	<0.001	0.427	0.154	<0.001	0.981	0.066
Protease	0.897	0.880	0.839	0.417	0.818	0.581	0.840
Interactions							
B x Phyt	0.292	0.307	0.269	0.550	0.249	0.074	0.077
B x Xyl	0.802	0.773	0.743	0.500	0.477	0.365	0.155
B x Prot	0.338	0.306	0.369	0.053	0.614	0.301	0.864
Phyt x Xyl	0.172	0.183	0.308	0.582	0.110	0.210	0.397
Phyt x Prot	0.624	0.697	0.134	0.666	0.391	0.033**	0.340
Xyl x Prot	0.982	0.980	0.556	0.766	0.962	0.085	0.029***
Phyt x Xyl x Prot	0.910	0.973	0.288	0.839	0.635	0.453	0.580
B x Phyt x Xyl	0.620	0.722	0.011*	0.583	0.739	0.760	0.549
B x Phyt x Prot	0.597	0.563	0.916	0.715	0.568	0.059	0.470
B x Xyl x Prot	0.471	0.523	0.239	0.538	0.434	0.575	0.184
B x Phyt x Xyl x Prot	0.648	0.609	0.752	0.576	0.583	0.841	0.195

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; NR, coefficient of total tract nitrogen retention; FD, coefficient of total tract fat digestibility; DMR, coefficient of total tract dry matter retention; ND, coefficient of ileal nitrogen digestibility; DMD, coefficient of ileal dry matter digestibility; Each value represents the mean of nine replicate pens of two birds each; Dietary AME, AMEn, CPR, FD, DMR, CPD and DMD variables were determined between 17 and 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$. [a,b]Values within a column with different superscripts differ significantly.

Xylanase supplementation increased AME (P=0.002), AMEn and DMR (P<0.001) by 0.18 MJ/kg DM and 1%, respectively, but did not affect (P>0.05) ND, NR, DMD and FD of the diets. Protease supplementation did not cause any alteration (P>0.05) of any of the

determined variables. Cultivar Divine tended to (P>0.05) respond better to exogenous enzyme supplementation. There was a bean cultivar by phytase by xylanase interaction (P<0.05) in NR, suggesting that with phytase alone there may be an improvement, although xylanase or a combination of xylanase and phytase did not change (P>0.05) NR in cultivar Divine (Table 4. 4 A). Additionally, the interaction of protease with phytase reduced (P<0.05) ND (Table 4. 4 B) and with xylanase decreased (P<0.05) DMD (Table 4. 4 C).

***Table 4. 4 A.** Interactions of bean, phytase and xylanase on NR (SEM = 0.0059) (see Table 4. 4)

	Phytase	No	No	Yes	No
Bean	Xylanase	No	Yes	No	Yes
Buzz		0.675	0.673	0.670	0.673
Clipper		0.676	0.665	0.679	0.678
Divine		0.672	0.688	0.701	0.680

****Table 4. 4 B.** Interaction of phytase and protease on ND (SEM = 0.0042) (see Table 4. 4)

Phytase	Protease	No	Yes
No		0.708	0.719
Yes		0.727	0.720

*****Table 4. 4 C.** Interaction of xylanase and protease on DMD (SEM = 0.0038) (see Table 4. 4)

Xylanase	Protease	No	Yes
No		0.621	0.629
Yes		0.637	0.628

4. 3. 3. Effect of the experimental diets on digestive tract components of the birds

Data on gastrointestinal tract responses to the experimental diets is summarised in Table 4. 5, and interactions in Tables 4. 5 A and 4. 5 B.

The overall weight of the total GIT, jejunum and ileum as percentage of total body weight was not different (P>0.05) by field bean cultivar. Inclusion of xylanase resulted in a reduction in pancreas relative weight % by 20% and an increase in PG% by 3.2%. Phytase reduced (P<0.05) the weight of the duodenum by 7.7%.

Table 4. 5. Effect of experimental diets on the gastrointestinal tract components of broiler chickens (data presented as a percentage of the live body weight)

Treatment factor	Total GIT	PG	Pancreas	SI	Duodenum	Jejunum	Ileum
Beans (B)							
Buzz	8.5	3.2	0.4	4.9	1.2	2.2	1.4
Clipper	8.4	3.2	0.4	4.8	1.2	2.2	1.4
Divine	8.3	3.1	0.4	4.7	1.2	2.2	1.3
SEM	0.08	0.04	0.01	0.07	0.02	0.04	0.02
Enzyme							
Phytase (Phyt)							
No	8.4	3.1	0.4	4.9	1.3	2.2	1.4
Yes	8.3	3.2	0.4	4.7	1.2	2.2	1.4
Xylanase (Xyl)							
No	8.4	3.1	0.5	4.8	1.2	2.2	1.4
Yes	8.4	3.2	0.4	4.8	1.2	2.2	1.4
Protease (Prot)							
No	8.3	3.1	0.4	4.7	1.2	2.2	1.3
Yes	8.5	3.2	0.4	4.8	1.2	2.2	1.4
SEM (df=181)	0.07	0.03	0.01	0.06	0.02	0.03	0.02
P values							
Beans	0.126	0.236	0.776	0.231	0.184	0.460	0.424
Phytase	0.256	0.105	0.553	0.041	0.010	0.086	0.477
Xylanase	0.773	0.022	0.005	0.635	0.826	0.486	0.977
Protease	0.133	0.085	0.735	0.418	0.528	0.806	0.156
Interactions							
B x Phyt	0.829	0.978	0.043*	0.907	0.272	0.605	0.803
B x Xyl	0.754	0.854	0.118	0.689	0.893	0.727	0.595
B x Prot	0.212	0.055	0.292	0.813	0.755	0.903	0.078
Phyt x Xyl	0.089	0.295	0.207	0.203	1.000	0.065	0.494
Phyt x Prot	0.259	0.586	0.957	0.115	0.054	0.454	0.108
Xyl x Prot	0.430	0.412	0.597	0.670	0.366	0.997	0.682
Phyt x Xyl x Prot	0.393	0.184	0.509	0.117	0.925	0.073	0.076
B x Phyt x Xyl	0.450	0.919	0.617	0.400	0.355	0.425	0.237
B x Phyt x Prot	0.067	0.034**	0.095	0.426	0.613	0.682	0.307
B x Xyl x Prot	0.153	0.086	0.939	0.410	0.102	0.379	0.274
B x Phyt x Xyl x Prot	0.906	0.181	0.262	0.543	0.260	0.852	0.683

GIT, gastrointestinal tract (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard; SI, small intestine (including duodenum, jejunum and ileum); Each value represents the mean of nine replicate pens; Gastrointestinal tract components were measured at 21 days of age using the heavier bird in each pen; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$.

There was a bean by phytase interaction ($P<0.05$) on the development of the pancreas, showing an increase of 25% in the pancreas of birds fed Divine and phytase (Table 4. 5 A). Further interaction ($P<0.05$) on the PG development was observed between beans, phytase and protease (Table 4. 5 B).

***Table 4. 5 A.** Interaction of bean and phytase on the pancreas% (SEM = 0.01) (see Table 4. 5)

Bean	Phytase	No	Yes
Buzz		0.4	0.4
Clipper		0.4	0.4
Divine		0.4	0.5

****Table 4. 5 B.** Interactions of bean, phytase and protease on PG% (SEM = 0.20) (see Table 4. 5)

	Phytase	No	No	Yes	No
Bean	Protease	No	Yes	No	Yes
Buzz		3.1	3.2	3.3	3.2
Clipper		3.2	3.1	3.2	3.3
Divine		3.0	3.2	3.0	3.3

4. 4. Discussion

The objective of the reported experiment was to investigate the responses of broiler chickens growth performance, ND, total tract nutrient retention and ME with the addition of phytase, xylanase, and protease individually or in combinations to wheat-soybean diets containing 300 g/kg of one of three different field bean cultivar samples. The ME and protein contents of the diets were either similar to or exceeding breeder's recommendation. The dietary mineral content was similar between diets and in keeping with recommendations. Therefore, the design allowed an examination of the effects of different field bean cultivars inclusion to the diet or the supplementation of the three enzymes individually or in combination.

4. 4. 1. Effect of the field bean cultivars

Non-starch polysaccharides and phytic acid in field beans can form strong complexes with proteins, starch, and minerals (Lekha and Lonsane, 1997; Bedford, 2006). Thus, the observed higher weight gain, feed efficiency, dietary AME, and nitrogen and dry matter utilisation of birds fed Divine (the bean sample with the lowest anti-nutrient content) over those fed Buzz and Clipper-containing diets is not a surprise. Also in the previous study, slightly better broiler performance, energy and nutrient availability was found with Divine compared to those of the other two bean cultivars, particularly Buzz, but the differences in the previous study were smaller than those in this study. The greater differences in the variables between the cultivars in the current experiment than those of the same cultivar samples in the previous study may be due to using a higher proportion of beans in this study (300 g/kg diet) than the previous one (200 g/kg diet), in addition to variation in the

composition of the balancer feeds used in the both studies. Additionally, the current study had higher residual degrees of feedom than the previous one (181 *versus* 63), increasing the sensitivity of the analysis. The reported results are in line with the observed improved ME, nutrient digestibility and growth performance of broilers when fed low anti-nutrients compared to high anti-nutrient diets (Kalmendal *et al.,* 2011; O'Neill *et al.,* 2012). Cowieson *et al.* (2003) also explained changes in growth performance of birds fed pea diets with differences in the carbohydrate fraction of dietary pea. In contrast, Nalle (2009) did not find significant differences in broilers' performance when fed four different bean cultivars with an NSP range similar to that reported in this study. However, the relatively low inclusion rate of 20% beans may be the reason for the results reported by Nalle (2009).

The inclusion of different field bean cultivars did not alter the component weights% of the GIT segments, although there were few interactions with the exogenous enzymes. Nalle (2009) also did not find a significant difference in the relative empty weights of most of the GIT segments for 21 day old male Ross 308 birds, fed maize based diets containing 200 g/kg of different field bean cultivars. The bean with phytase interaction produced enlarged pancreas in Divine fed birds coupled with the improved NR. The secretion of pancreatic enzymes might be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine following a negative feedback mechanism (Kubena *et al.,* 1983). Mahagna *et al.* (1995) reported that secretion of pancreatic amylase and proteases was reduced when chicks were fed diets supplemented with the same enzymes. If the efficiency of digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction of anti-nutritive factors, the GIT responds by increasing both size (surface area) and digestive enzyme output (Bedford, 2006). The relationship between phytic acid and protein utilisation is well documented (Cowieson *et al.,* 2004; Pirgozliev *et al.,* 2009). A reduction in phytate concentration due to exogenous phytase supplementation should enable better utilisation of CP, AAs and other nutrients in young birds. The release of nutrients in the lumen of the small intestine, due to phytase supplementation, would lead to a release of more enzymes from the pancreas, thus explaining the enlargement of the pancreas in relation to phytase.

The observed interaction between beans, phytase and protease on the PG% suggests the cultivar Divine contains fewer anti-nutrients, as it showed less pronounced and more random responses to exogenous enzymes. Regarding GIT development, however, compared to other reports on GIT development (Gracia *et al.,* 2003; Wu *et al.,* 2004; Nalle, 2009), the observed differences are relatively small and may not represent changes in the functions of organs.

4. 4. 2. Effect of the enzymes

In this study, phytase gave the best improvement as a single enzyme, but the responses to single xylanase supplementation were less pronounced. There was, however, no detectable response to protease when fed singularly. A further additive effect on feed intake and weight gain was observed when both phytase and xylanase and the three enzymes were fed together. Interactions between protease and phytase on ND, and protease and xylanase on DMD, showing a slight reduction of the coefficients with protease addition, were observed.

Effects of dietary enzyme combination on broiler growth performance and nutrient utilisation are frequently inconsistent. Although some authors reported antagonism between different enzymes (Naveed *et al.,* 1999; Saleh *et al.,* 2004), while others found the effects of enzyme combinations to be subadditive (Wu *et al.,* 2004; Kim *et al.,* 2005), additive (Romero *et al.,* 2014; Olukosi *et al.,* 2015), and synergistic (Ravindran *et al.,* 1999). Differences in the type of enzymes tested, as well as in the experimental design, especially the dietary composition and the use of enzyme mixtures rather than monocomponent enzyme preparations, can partially explain the conflicting and highly variable results reported (Cowieson and Ravindran, 2008; Angel *et al.,* 2011; Walk *et al.,* 2011). When a few enzymes are tested together the effect of the enzyme preparation cannot always be attributed to the addition of a specific enzyme.

The positive effect of phytase on available protein, mineral retention and broiler growth on low phosphorus diets is well documented (Cowieson *et al.,* 2004; Selle and Ravindran, 2007; Pirgozliev and Bedford, 2013). Phytases (*myo*-inositol hexaphosphate phosphohydrolases) are enzymes that are able to hydrolyse the ester bonds between the phosphate groups and the inositol ring in phytates, increasing the dietary available phosphorus (Nelson *et al.,* 1971; Irving and Cosgrove, 1974). Phytate is also an irritant that increases the endogenous losses from the broiler's GIT (Cowieson *et al.,* 2004; Pirgozliev *et al.,* 2012), thus hydrolysing dietary phytates will further increase the benefits of supplementary phytase. The diets in the reported study contained less than the recommended level of available phosphorus (4.4 g/kg diet), thus partly explaining the improvements in the determined performance, ND and DMD variables due to phytase supplementation. Breaking down the inherent dietary phytate binary complexes (phytate-protein complexes) and preventing the formation of phytate ternary complexes (phytate-mineral-protein complexes) in the digestive tract of birds (Selle *et al.,* 2000; Cowieson *et al.,* 2004), prevents endogenous losses in the form of enzymes, by hyperactive pancreas. This may have been an additional cause of the observed improvements (particularly in ND) in the current study, following phytase addition.

The benefits of using fibre-degrading enzymes in broiler feed are associated with reducing intestinal viscosity, degradation of cell wall NSPs and the releasing of previously encapsulated nutrients by cell wall materials in the gut (Choct *et al.,* 2006). The

experimental field beans, representing 30% of the experimental diets, on average contained 21% of NSP, thus explaining the benefits observed in increasing ME and DMR, in line with a reduction in pancreas enlargment in this study when xylanase was supplemented in the diets.

Although, the birds cannot produce xylanase and sufficient amounts of phytase in their gastrointestinal tract, the wide range of endogenous proteases synthesised and released in the GIT is generally considered to be sufficient to optimise feed protein utilisation (Le Heurou-Luron *et al.,* 1993; Nir *et al.,* 1993). This may be the reason for the lack of significant effects following supplementation of protease singularly on the measured parameters in the current study. However, nitrogen and AA digestibilities reported for poultry indicate that valuable amounts of protein pass through the GIT without being completely digested (Parsons *et al.,* 1997; Wang and Parsons, 1998; Lemme *et al.,* 2004). This undigested protein represents an opportunity for the use of supplemental exogenous proteases in broiler feeds to improve protein digestibility (Angel *et al.,* 2011). Improvements in nutrient availability and performance of broilers have been reported following supplementation of exogenous proteases alone and in a combination with other exogenous enzymes, such as phytase and xylanase (Olukosi *et al.,* 2010, 2015; Romero *et al.,* 2014).

Results from *in vitro* studies have shown that phytate limits nutrient availability in field beans and phytase alone or in combination with carbohydrase (cellulase) additively increases their relative nutrient availability (Luo *et al.,* 2010, 2012). Xylanase increases the access of proteolytic enzymes to substrates, e.g. phytate and protein, in the aleurone layer of wheat (Parkkonen *et al.,* 1997), suggesting that xylanase may increase the access of the enzymes to substrates by disrupting the cell wall matrix. Frolich (1990) stated that phytic acid is not the component that is solely responsible for the decreased nutrient availability in diets, as dietary fibre itself also might be of importance. Thus, phytase might also assist the hydrolytic functions of xylanase by dephosphorylation of phytate. Colombatto and Beauchemin (2009) also demonstrated in ruminant models the ability of exogenous proteases to increase the solubilisation and fermentation of hemicellulose by removing structural proteins in the cell wall, allowing faster access of ruminal microbes to digestible substrates. All this supports the observed improvements in FI and WG of birds fed a combination of xylanase, phytase, and protease compared to the effects of feeding a single enzyme. Although statistically significant, the discrepancies in ND following a combination of protease with phytase and DMD due to the combination of protease and xylanase were relatively small with limited biological value.

4. 5. Conclusion

In conclusion, the results of the current study showed that the phytic acid and NSP contents negatively influence the feeding value of field beans for broiler chickens. Supplementation of phytase alone can improve performance and protein utilisation, and xylanase alone can improve the ME of field bean-containing diets for broiler chickens. The use of a combination of phytase and xylanase together and these with the addition of protease improved FI and WG of birds. However, an interaction between the enzymes and bean cultivar samples was observed, thus showing that the activity of the enzymes may depend on bean composition. Since the improvements of the feeding quality from supplementation of these enzymes individually and in combination in this study were not practically important for the broiler feed industry, investigating the impact of other potential benifical enzymes on the nutritional value of field bean-based diets is needed. Since tannins reduce the energy and nutrient availability of field beans for broilers, examination of the impact of tannase enzyme on the nutritional quality of a high tannin field bean-based diet for growing broilers is needed.

CHAPTER 5: NUTRITIONAL VALUE OF DIETS BASED ON SULTAN FIELD BEAN CULTIVAR WITH AND WITHOUT SUPPLEMENTATION OF AN ENZYME MIXTURE CONTAINING TANNASE, PECTINASE AND XYLANASE ACTIVITIES FOR BROILERS; A PRELIMINARY STUDY

5. 1. Introduction

It is well documented that field bean tannins reduce the energy and nutrient availability of field beans for growing broilers (Marquardt and Ward, 1979; Vilariño *et al.,* 2009; Woyengo and Nyachoti, 2012). Results from the study reported in chapter three showed that Sultan field beans had the lowest availability of energy, all nutrients and also led to the lowest broiler performance amongst the ten studied UK field bean cultivars. The lowest nutritive value was paralleled with the highest total phenolic compounds, tannins (as tannic acid equivalents) and condensed tannins (as leucocyanidin equivalents), in addition to high NSP and the lowest AA contents in this field bean cultivar.

It was ascertained from the study in chapter four that the inclusion of phytase, xylanase and protease singularly or in combinations did not lead to economically important improvements in the nutritive value of field bean-based diets for broiler chickens. There is a lack of information on the effect of tannin degrading enzymes on the feeding value of high tannin-containing field beans for broilers. Therefore, the main objective of this experiment was to investigate the influence of a tannase-containing enzyme product on dietary energy and nutrient availability, growth performance and gastrointestinal tract components, when a diet containing Sultan (at 300 g/kg) was fed to 7 to 21 day-old broiler chickens. Due to the unavailability of a purified tannase enzyme preparation (having only tannase activity) on the market, this multi enzyme preparation, which had tannase and other enzyme activities, was tested in this feeding experiment.

5. 2. Materials and methods

5. 2. 1. Dietary treatments

A control diet containing 300 g/kg of the freshly hammer-milled (using a 4 mm screen) Sultan field bean sample was prepared (Table 5. 1). The diet was then split into two batches and one of them was supplemented with 3400 units/kg (TU) of propriety tannase (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in a tannase supplemented diet. The enzyme had also 6220 units/kg diet of pectinase and less than 200 units/kg diet of phytase activity. The enzyme preparation was based on tannase biosynthesised from *Aspergillus niger*. The enzyme was in a liquid form and the reported enzyme activities were obtained after spraying 17 ml/kg on top of the diet. The DM content of control diet was adjusted by spraying of 17 ml water per kg of diet. After spraying, each of the diets were thoroughly mixed in a horizontal mixer and fed as mash.

Diets were free of coccidiostat, antimicrobial growth promoters, prophylactic and other similar additives.

Table 5. 1. Ingredient composition of the experimental broiler chicken diet formulations

Ingredient	g/kg as-fed
Wheat meal	404.2
SBM (CP=48%)	27.0
Full-fat soybean meal	127.5
Maize gluten meal	35.0
Sultan field bean	300.0
Soya oil	65.0
Lysine HCL	2.3
$_{DL}$-Methionine	5.8
$_{L}$-Threonine	2.4
Monocalcium phosphate	10
Limestone	14.0
Sodium chloride	2.8
Vitamin/mineral premix*	4.0
Total	1000
Determined energy and nutrient composition	
Gross energy (MJ/kg DM)	18.34
Protein (g/kg)	171
Fat (g/kg)	132
Dry matter (g/kg)	885
Calculated energy and nutrient composition	
ME (MJ/kg)	12.65
Protein (g/kg)	212
Lysine (g/kg)	12.4
Methionine + cysteine (g/kg)	9.4
Calsium (g/kg)	8.2
Non-phytate phosphorus (g/kg)	4.0

Vitamin and mineral premix provided per kg diet: 30 mg niacin, 25 mg α-tocopherol, 8 mg pantothenic acid, 5 mg riboflavin, 2.16 mg retinol, 1.5 mg menadione, 1.5 mg pyridoxine, 1.5 mg thiamine, 0.5 mg folic acid, 75 μg cholecalciferol, 60 μg biotin, 10 μg cyanocobalamin, 80 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 0.8 mg iodine and 0.3 mg selenium; Diets were not supplemented with coccidiostat.

5. 2. 2. Bird husbandry, experimental design and sample collection

The experiment was approved by Research Ethics Committee at Harper Adams University.

Eighty male Ross 308 broiler chicks were obtained from a commercial hatchery at one-day old and were placed in a single floor pen and fed on a proprietary broiler starter feed until 6 days of age. On the first day of the experimental period (at 7 days of age), the chicks were individually weighed and randomly placed in one of the experimental pens. Two birds were placed in each pen (0.16 m^2 solid floor area) within a controlled

environment. Each diet was fed to 16 pens of birds from 7 to 21 days of age, in a randomised block design, resulting in 32 pens of birds. Information on daily feed intake (FI), daily weight gain (WG) and feed conversion ratio (FCR) was obtained over the same period. The room temperature and lighting programme followed breeder's recommendations (Aviagen Ltd., Edinburgh, UK). Access to the feed and the water was *ad libitum* throughout the experimental period.

During the last four days of the study, from 17 to 21 days of age, the solid floor of each pen was replaced with a wire mesh and all excreta output were quantitatively collected. Feed intakes were also measured for the same period. Collected excreta were immediately oven-dried in a forced draft oven at 60°C until a constant weight, weighed after leaving at room temperature for three nights, and then milled to pass through a 0.8 mm mesh.

On the final day of the experiment, at 21 days of age, the two birds in each pen were killed by cervical dislocation and then weighed. The empty weights of gastrointestinal tract segments, including proventriculus and gizzard (PG), pancreas, duodenum, jejunum and ileum of the heavier bird in each pen were taken according to the procedures described by Amerah and Ravindran (2008). The heavier bird in each pen was selected for measuring GIT segments, as it was considered to be more responsive to the dietary treatments. The weights of the gastrointestinal segments were presented as absolute weight and relative to live body weight (BW%).

The GE, DM, nitrogen and oil in feed and excreta were determined as explained previously for the field bean sample (see Section 2. 2. 2. 1). Dietary apparent metabolisable energy (AME) and nitrogen-corrected apparent metabolisable energy (AMEn) were calculated as described by Hill and Anderson (1958). The coefficients of total tract dry matter (DMR) and nitrogen retention (NR), and total tract fat digestibility (FD) were determined as the difference between the respective nutrient intake and nutrient excreted divided by its intake as described earlier (see Section 3. 2. 3).

5. 2. 3. Statistical analyses

Statistical analyses were performed using the Genstat statistical software package (Genstat 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The metabolisable energy, DMR, NR and FD content of the experimental diets, broiler growth performance variables and the empty and relative to body weights of gastro intestinal tract segments of the birds were compared statistically by one-way ANOVA. In all instances, differences were reported as significant at $P \leq 0.05$. Tendencies towards significance ($P \leq 0.10$) were also reported.

5. 3. Results

All birds were healthy throughout the studying period and there was no mortality. There was no effect (P>0.05) of treatments on daily weight gain (WG) of the birds (Table 5. 2). The overall final body weight of the birds was 742 g (not presented in the table). Feeding tannase-supplemented diet to birds reduced (P<0.05) daily feed intake (FI) but improved (P<0.05) feed conversion ratio (FCR) compared to the control diet (by 4.4 and 2.6%, correspondingly).

Exogenous tannase supplementation improved dietary AME and AMEn by 0. 42 and 0.40 MJ/kg DM, respectively, compared to the control (P<0.05) (Table 5. 3). Similarly, tannase supplementation resulted in an improvement in NR (P<0.001), DMR and FD (P<0.05), by 3.2, 2.8 and 6.5%, respectively. Birds fed tannase had reduced absolute and relative to body weights of pancreas and PG compared to birds fed the control diet (P<0.05) (Table 5. 4, 5. 5). There was no effect (P>0.05) of dietary treatment on the absolute and relative to body weights of total GIT, duodenum, jejunum, ileum and small intestine of the broiler chickens.

Table 5. 2. Effect of experimental diets on growth performance of broilers

Treatments	FI (g DM/b/d)	WG (g/b/d)	FCR (g:g)
Control	59.0	53.5	1.104
Tannase	56.4	52.4	1.076
SEM (df=31)	0.74	0.50	0.0083
P value	0.025	0.165	0.031

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; Each mean represents values from sixteen replicate pens of two birds each; Bird performance was determined from 7 to 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$.

Table 5. 3. Energy and nutrient availability of the experimental diets for broilers

Treatments	AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR
Control	13.93	13.20	0.634	0.744	0.641
Tannase	14.35	13.60	0.654	0.792	0.659
SEM (df=31)	0.081	0.079	0.0033	0.0107	0.0039
P value	0.002	0.003	<0.001	0.007	0.006

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; NR, coefficient of total tract nitrogen retention; FD, coefficient of total tract fat digestibility; DMR, coefficient of total tract dry matter retention; Each mean represents values from sixteen replicate pens of two birds each; Dietary AME, AMEn, NR, FD and DMR variables were determined between 17 and 21 days of age; SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$.

Table 5. 4. The effect of experimental diets on the gastrointestinal tract components of broiler chickens

Treatments	Total GIT (g)	PG (g)	Pancreas (g)	SI (g)	Duodenum (g)	Jejunum (g)	Ileum (g)
Control	75.43	26.43	3.75	45.25	11.16	19.42	14.67
Tannase	72.20	24.06	3.44	44.70	10.80	19.04	14.86
SEM (df=31)	1.282	0.491	0.072	1.049	0.281	0.430	0.527
P value	0.095	0.004	0.007	0.718	0.378	0.545	0.801

GIT, gastrointestinal tract weight (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard; SI, small intestine weight (including duodenum, jejunum and ileum); Each mean represents values from sixteen replicate pens; Gastrointestinal tract components were measured at 21 days of age using the heavier bird in each pen. SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$.

Table 5. 5. The effect of experimental diets on the gastrointestinal tract components of broiler chickens (data presented as a percentage of the live body weight)

Treatments	Total GIT %	PG %	Pancreas %	SI %	Duodenum %	Jejunum %	Ileum %
Control	9.39	3.29	0.47	5.64	1.39	2.42	1.83
Tannase	9.26	3.09	0.44	5.73	1.38	2.44	1.90
SEM (df=31)	0.102	0.048	0.009	0.104	0.033	0.051	0.054
P value	0.363	0.012	0.042	0.565	0.855	0.748	0.360

GIT%, gastrointestinal tract (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG%, proventriculus and gizzard; SI%, small intestine (including duodenum, jejunum and ileum); Each mean represents values from sixteen replicate pens; Gastrointestinal tract componnts were measured at 21 days of age using the heavier bird in each pen. SEM, standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$.

5. 4. Discussion

The present study evaluated the efficacy of supplementary tannase enzyme on growth performance, energy and nutrient utilisation, and GIT development when a high tannin field bean-containing diet was fed to broilers. The data demonstrates that young broilers were sensitive to dietary supplementation with exogenous tannase. There is no published study showing the impact of dietary tannase supplementation in broiler diets. However, there are published reports on the negative impact of high dietary tannin on the studied variables (Jansman, 1993; Brufau *et al.,* 1998; O'Neill *et al.,* 2012).

The diets were relatively high in their tannin contents from the Sultan field bean cultivar inclusion; approximately 2.54 and 1.86 g/kg diet as tannic acid and leucocyanidin equivalents, respectively. All growth performance parameters were comparable to those

found with birds fed a Sultan-based diet in the study reported in chapter three. Also, the growth of the birds in this study did not differ between diets and was in the expected range for broilers reared in a similar environment and fed mash diets (Karadas *et al.,* 2014; Pirgozliev *et al.,* 2015a, b). In agreement with improved ME and nutrient utilisation, birds fed a tannase supplemented diet had an improved FCR. This is in line with Marquardt and Ward (1979) and Kubena *et al.* (1983) who reported reduced feed efficiency when high tannin diets were fed to poultry.

Tannins are able to form complexes with proteins, so they can also bind to enzymes, which have implications for their biological activity. It has been reported that high-tannin inclusion reduces the activities of all digestive enzymes in various *in vitro* and *in vivo* assays (Griffiths, 1979; Griffiths and Moseley, 1980; Singh, 1984). Thereby, the increased nutrient utilisation coefficients in the recent report suggesting that exogenous tannase was able to hydrolyse at least a part of the dietary tannins and alleviate their negative impact observed in other studies. This is in line with the observed improved AME, nitrogen and AA digestibility of broilers when fed diets low in tannin compared to high tannin field bean-containing diets (Nyachoti *et al.,* 1996; Metayer *et al.,* 2003; Vilariño *et al.,* 2009; Woyengo and Nyachoti, 2012).

Kubena *et al.* (1983) and Ahmed *et al.* (1991) also found an increased pancreas in broilers fed high-tannin diets. Similar to trypsin inhibitors, tannins are also able to form complexes with proteins and bind to enzymes. Therefore, tannins may stimulate pancreatic secretion in a manner analogous to that of proteinase inhibitors from legume seeds (Griffiths, 1980), suggesting an explanation for the reduced pancreas size in birds fed tannase in this study.

Kubena *et al.* (1983) found that the weights of PG of birds fed high tannin feed were lower compared to the control fed birds. This conflicts with the present findings that reducing dietary tannin (via supplementing diets with tannase) reduced PG weights of birds. In the present study the diets contained about 2.54 g/kg tannins (measured as tannic acid), although Kubena *et al.* (1983) had 15 g tannic acid per kg diet.

5. 5. Conclusion

Supplementation of a field bean-based diet with tannase enzyme improved feed efficiency, dietary ME and nutrient utlisation. Although the beneficial effects associated with tannase treatment were in line with the reduction of pancreas size, it is possible that the pectinase activity in the tannase preparation may have also affected the responses of the birds. The functional mechanism of this enzyme preparation requires further investigation by examining its impact on other parameters in addition to those measured in this study, for broilers fed diets containing different tannin containing field bean cultivars along with a tannin free diet.

CHAPTER 6: FEEDING VALUE FOR CHICKS OF FIELD BEANS (*Vicia faba* L. var. *minor*) WITH AND WITHOUT ENZYME CONTAINING TANNASE, PECTINASE AND XYLANASE ACTIVITIES

6.1. Introduction

Results from the previous study indicated that supplementation of the exogenous tannase-containing enzyme can significantly improve the feeding value of a high tannin Sultan field bean cultivar-based diet for broiler chickens. However, there is no information on the effect of the enzyme product on diets with different field bean samples (with different tannin concentrations) and in comparison with other low-tannin diets. Therefore, the main objective of this experiment was to determine the effect of a supplementary tannase-containing enzyme preparation on the dietary energy and nutrient utilisation, growth performance, ileal digesta viscosity, mucin losses (as sialic acid secretion in excreta), gastrointestinal tract components and jejunul villus morphometry, when diets containing field beans with different tannin contents were fed to 14 to 21 day-old broiler chickens.

6. 2. Materials and methods

6. 2. 1. Dietary treatments

A control diet was prepared with the main ingredients of 400.0 g/kg wheat and 127.0 g/kg full fat SBM, and contained 221 g/kg CP and 12.83 MJ/kg ME in agreement with the breeder's recommendation (Aviagen Ltd., Edinburgh, UK) (Table 6. 1). To reduce nutrient density, the control diet also contained 119.1 g/kg washed sand. Three more diets containing 300 g/kg of one of three experimental freshly hammer-milled (passing a 4 mm screen) field bean cultivar samples (Maris Bead, Sultan and Wizard) in replacement for SBM and sand were also mixed in order to have ME and CP in a range similar to the control diet (Table 6. 1). Each diet was then split into two batches and one of them was supplemented with an enzyme mixture (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in eight mash diets in total. The determined enzyme activities of the enzyme mixture were; tannase or tannin acyl-hydrolase (E.C. 3.1.1.20) 3400 units/kg diet (following the method of Bajpai and Patil (1996) at pH 5.5; determined by Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland), pectinase (EC 3.2.1.15) 6220 units/kg diet (ESC Standard Analytical Method SAM027 at pH 4.5 and 40°C; determined by Enzyme Services & Consultancy, Ystrad Mynach, UK); xylanase (EC 3.2.1.8) 6100 units/kg diet (ESC Standard Analytical Method SAM036 at pH 5.3 and 50°C, using 1.2% BSA in the extraction; determined by Enzyme Services & Consultancy, Ystrad Mynach, UK), and there were some additional amylase and α-galactosidase activities. The enzyme mixture was synthesised by *Aspergillus niger*. The enzyme was in a liquid form

and the reported enzyme activities were obtained after spraying 17 ml/kg on top of the diets. The dry matter content of non-supplemented diets was adjusted by spraying of 17 ml water per kg of diet. After spraying, each diet was thoroughly mixed in a horizontal mixer. Diets were free of coccidiostat, antimicrobial growth promoters, prophylactic and other similar additives.

Table 6. 1. Ingredient composition (g/kg, as-fed) of the experimental broiler chicken diet formulations

Ingredient	Control	Maris bead	Sultan	Wizard
Wheat	400.0	404.2	404.2	404.2
Bean (Maris Bead)	-	300.0	-	-
Bean (Sultan)	-	-	300.0	-
Bean (Wizard)	-	-	-	300.0
SBM (CP=48%)	190.4	27.0	27.0	27.0
Full fat soybean meal	127.0	127.5	127.5	127.5
Maize gluten meal	35.0	35.0	35.0	35.0
Washed sand	119.1	-	-	-
Soya oil	82.5	65.0	65.0	65.0
Lysine	6.0	2.3	2.3	2.3
Methionine	6.8	5.8	5.8	5.8
Threonine	2.4	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0	10.0
Limestone	14.0	14.0	14.0	14.0
Salt	2.8	2.8	2.8	2.8
Vitamin/mineral premix*	4.0	4.0	4.0	4.0
Total	1000	1000	1000	1000
Analysed values (as-fed)				
DM	855	877	876	876
GE (MJ/kg)	16.21	17.57	17.52	17.60
CP	197	198	183	197
Fat	112	95	95	95
Total phenols[a]	1.312	2.770	3.791	3.084
Tannins[a]	0.452	1.991	2.550	2.159
Condensed tannins[b]	0.00	1.17	1.86	1.53
Calculated values				
ME (MJ/kg)	12.83	13.12	12.65	13.15
Crude protein (g/kg)	221	217	201	216
Fat (g/kg)	113	97	97	97
Calsium (g/kg)	7.9	8.1	8.2	8.2
Available phosphorus (g/kg)	4.4	4.4	4.4	4.4
Total lysine (g/kg)	15.1	12.4	11.8	12.7
Total methionine + cysteine (g/kg)	13.5	8.6	8.4	8.6

*Vitamin and mineral premix provided per kg diet: 30 mg niacin, 25 mg α-tocopherol, 8 mg pantotenic acid, 5 mg riboflavin, 2.16 mg retinol, 1.5 mg menadione, 1.5 mg pyridoxine, 1.5 mg thiamine, 0.5 mg folic acid, 75 *µ*g cholecalciferol, 60 *µ*g biotin, 10 *µ*g cyanocobalamin, 80 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 0.8 mg iodine and 0.3 mg selenium; Diets were not supplemented with coccidiostat; [a] As tannic acid equivalent; [b] As leucocyanidin equivalent.

6. 2. 2. Animal husbandry, experimental design, determination of dietary metabolisable energy, nutrient utilisation and broiler growth performance

The experiment was approved by Research Ethics Committee at Harper Adams University.

One hundred and sixty day-old male Ross 308 broiler chicks were obtained from a commercial hatchery. During the pre-study period, from day old to 13 days of age, the birds were reared in a single floor pen and fed a proprietary wheat-based diet without coccidiostats or antimicrobial growth promoters, prophylactic or other similar additives. At the beginning of the study, at 14 days of age, 144 chicks were allocated to 72 small pens with a 0.16 m^2 solid floor area, two birds in each pen. Room temperature and lighting programme followed breeder's recommendations (Aviagen Ltd., Edinburgh, UK). Feed and water were offered *ad libitum* to birds throughout the experiment. Each diet was offered to birds in 9 pens in a randomised block design. Information on daily feed intake (FI), daily weight gain (WG) and feed conversion ratio (FCR) was obtained from 14 to 21 days of age. During the last four days of the experiment, from 17 to 21 days of age, the solid floor of each pen was replaced with a wire mesh and all excreta were collected and immediately dried at 60^{o}C, left at room temperature for three nights before being weighed and milled. Feed intake over the same period was also measured. The GE, DM, nitrogen, and fat of each dried excreta sample and the experimental diets were determined as described for the field bean samples (see Section 2. 2. 2. 1). The total phenols and total tannins in the control diet, as well as representative samples of the studied field bean cultivars, all as tannic acid equivalents, were determined by applying the procedure suggested by Makkar *et al.* (1993). Whereas condensed tannins (as leucocynidin equivalents) for the same samples were determined by using the assay described by Porter *et al.* (1985), as previously explained for the field bean samples (see Section 2. 2. 2. 7). The AME and AMEn of the diets were calculated as described by Hill and Anderson (1958). The coefficients of total tract fat digestibility (FD), dry matter (DMR) and nitrogen (NR) retention were determined as the difference between intake and excretion (retention) of each nutrient divided by its respective intake (see Section 3. 2. 3).

6. 2. 3. Determination of mucin losses (as sialic acid) in excreta

The mucin losses in excreta were measured using the concentration of the sialic (SA) as a marker applying the method described by Jourdian *et al.* (1971) as following: an excreta output sample of 20 mg was weighed into a 10 ml screw-capped plastic tube, 500 µl of previously made standard, 500 µl of deionised distilled water, and then 100 µl of previously prepared 0.04 M periodic acid (0.4559 g periodic acid (VWR Ltd, UK) in 50 ml distilled water) were respectively added to the tube. After vortexing of the mixture, the tube was placed in ice for 20 minutes. While the tube was still in the ice bath, 1.25 ml of resorcinol reagent (7 mg $CuSO_4.5H_2O$ (VWR, Ltd., UK), 0.6 g resorcinol granules, 40 ml

distilled water and 60 ml of 25% HCl) was added and then the tube was left in the same place for additional 5 minutes and the lid was put back on the tube. The tube was transferred to a previously prepared water bath and left for 15 minutes at 100°C. After cooling down with tap water, 1.25 ml of 95% tert-butyl alcohol (95 ml of 2-methyl-propan-2-ol (VWR Ltd, UK) and 5 ml distilled water) was added to the tube. The tube was kept in a 37°C water bath for 3 minutes to stabilise the colour, and then the tube contents were centrifuged (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) at 1500 x*g* for 5 minutes at 5^{o}C. The absorbance of standard and sample was read at 630 nm in a spectrophotometer (Beckman DU 650 spectrophotometer, Beckman instruments, INC. Fullerton, California, USA). This procedure detects total, free, and glycosidically bound N-acetyl neuraminic (sialic) acid. The mucin losses in excreta are presented in results and tables as SA.The total SA excretion was obtained by multiplying the SA concentration by the amount of dry excreta collected.

6. 2. 4. Ileal digesta viscosity

On the final day of the study, at 21 days of age, the two birds in each pen were killed by cervical dislocation and weighed. The ileal digesta from both birds in each pen were collected and pooled then centrifuged (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) at 10000 x*g* for 2 minutes. The viscosity of the supernatant (0.5 ml supernatant) (in centipoise (cP) units) was measured using a rotating cone and cup viscometer (model DV – II + LV, Brookfield Engineering Laboratories, USA), as described by Bedford and Classen (1992).

6. 2. 5. Gastrointestinal tract components and ileal villus morphometry

The relative empty weights of the GIT segments including PG, small intestine and pancreas of each bird were determined as previously described (Amerah and Ravindran, 2008; Pirgozliev *et al.,* 2016). After that, approximately 5 cm of the middle part of the jejunum, between the point of bile duct entry and Meckel's diverticulum, of one bird per cage was sampled and stored for 2 weeks in 10% neutral buffered formalin, after washing with normal saline. The samples then were embedded in paraffin wax, sectioned at approximately 5 µm, and 3 gut segments were fixed in each slide. Morphometric measurements were determined on 25 intact well-oriented villus–crypt units for each slide (microscope Microtec, TEC Microscopes LTD, Axbridge, UK; CCD camera Infinity 2, Lumen*era* Corporation, Ottawa, Canada; Image analysis software, Infinity Analyse – Infinity 2-2 for Windows version 6.5.2, Lumen*era* Corporation, Ottawa, Canada). The height and the width of the villus, and the crypt depth were determined as previously described (Viveros *et al.,* 2011). The distance between the bottom of the crypt and the outside of the intestine was measured and described as muscle thickness.

6. 2. 6. Statistical analysis

Statistical analyses were performed using the Genstat statistical software package (Genstat 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The studied variables were compared statistically by a two way ANOVA using a 2 × 4 factorial arrangement of treatments. The main effects were the enzyme supplementation and the four diet formulations (three bean samples and one control diet) giving a total of eight dietary treatments. The differences between the treatments means of the four diet formulations were separated using a Tukey's multiple range test. In addition, an orthogonal comparison contrast test was performed to compare the control diet with the mean of the three field bean diets and the interaction between bean cultivar and control with or without the exogenous enzyme supplementation. In all instances, differences were reported as significant at $P \leq 0.05$. Tendencies towards significance ($P \leq 0.10$) were also reported.

6. 3. Results

6. 3. 1. Effect of the experimental diets on growth performance of broilers chickens

Results on growth performance of the birds are shown in Table 6. 2. There were no mortalities, and the overall weight of the birds was 0.867 kg (data is not in tables), and in agreement with the breeder's recommendation (Aviagen Ltd, Edinburgh, UK) (Table 6. 2). Birds fed the control diet had higher ($P<0.001$) daily FI and WG, compared to the birds fed field beans containing diets. Diet containing the cultivar Sultan had a higher ($P<0.001$) FCR compared to the rest of the diets. Feeding the enzyme mixture supplemented diets tended ($P=0.069$) to reduce daily FI, and improved ($P<0.001$) dietary FCR by 3.3% compared to non-supplemented diets. An orthogonal comparison contrast test showed that birds fed bean containing diets had lower ($P<0.001$) FI and WG compared to the control, but no difference ($P>0.05$) was detected for FCR. There were no significant ($P>0.05$) diet x enzyme interactions.

6. 3. 2. Energy and nutrient availability of the experimental diets

The results on dietary available energy and nutrient utilisation are summarised in Table 6. 3. Sultan containing diets had relatively low metabolisable energy and nutrient utilisation coefficients compared to the rest of the field bean containing diets ($P<0.001$). Feeding the enzyme mixture improved ($P<0.001$) dietary AME and AMEn by 0.57 and 0.56 MJ/kg DM (4.0 and 4.1%), correspondingly. Enzyme supplementation also improved ($P<0.001$) DMR, NR and FD by 3.6%, 2.5% and 9.0%, respectively. The means of the three field bean diets for AME, AMEn and DMR were higher ($P<0.001$) and NR lower than the control diet. No differences ($P>0.05$) were observed for FD. There were no significant ($P>0.05$) diet x enzyme interactions.

Table 6. 2. Effect of experimental diets on growth performance of broilers

Treatment factor		FI (g DM/b/d)	WG (g/b/d)	FCR (g:g)
Diet				
Bean (Maris Bead)		75.8[a]	62.9[b]	1.206[a]
Bean (Wizard)		75.7[a]	61.4[ab]	1.234[a]
Bean (Sultan)		76.6[a]	58.5[a]	1.310[b]
Soybean meal (Control)		82.9[b]	67.3[c]	1.236[a]
SEM (df=56)		1.11	1.11	0.0110
Tannase				
No		78.8	62.3	1.268
Yes		76.7	62.8	1.226
SEM (df=56)		0.79	0.78	0.0078
Field bean cultivar or control diet	Enzyme			
Maris Bead	No	76.6	62.6	1.223
Maris Bead	Yes	75.1	63.1	1.190
Wizard	No	77.4	61.4	1.260
Wizard	Yes	74.0	61.4	1.208
Sultan	No	77.3	58.6	1.319
Sultan	Yes	75.8	58.3	1.302
Soybean meal	No	83.9	66.4	1.270
Soybean meal	Yes	81.9	68.3	1.202
SEM (df=56)		1.57	1.57	0.0155
P value				
Source		<0.001	<0.001	< 0.001
Tannase		0.069	0.649	< 0.001
Diet x Tannase interactions		0.921	0.890	0.383

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; Each mean represents values from nine replicate pens of two birds each; Bird performance was determined from 13 to 21 days of age; SEM, Standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$. [a,b,c]Values within a column with different superscripts differ significantly.

Table 6. 3. Energy and nutrient availability of the experimental diets for broiler chickens

Treatment factor		AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR
Diet						
Bean (Maris Bead)		14.95^{c}	14.10^{c}	0.649^{b}	0.744	0.655^{c}
Bean (Wizard)		15.01^{c}	14.16^{c}	0.657^{bc}	0.757	0.663^{c}
Bean (Sultan)		14.49^{b}	13.74^{b}	0.634^{a}	0.718	0.636^{b}
Soybean meal (Control)		13.96^{a}	13.12^{a}	0.660^{c}	0.750	0.606^{a}
SEM (df=56)		0.092	0.089	0.0031	0.0148	0.0051
Tannase						
No		14.32	13.50	0.642	0.710	0.632
Yes		14.89	14.06	0.658	0.774	0.648
SEM (df=56)		0.065	0.063	0.0022	0.0105	0.0036
Field bean cultivar or control diet	Enzyme					
Maris Bead	No	14.70	13.86	0.640	0.716	0.643
Maris Bead	Yes	15.21	14.34	0.659	0.772	0.667
Wizard	No	14.72	13.88	0.646	0.722	0.648
Wizard	Yes	15.30	14.43	0.668	0.792	0.677
Sultan	No	14.16	13.42	0.628	0.680	0.632
Sultan	Yes	14.82	14.07	0.639	0.756	0.641
Soybean meal	No	13.69	12.86	0.652	0.722	0.604
Soybean meal	Yes	14.24	13.38	0.668	0.778	0.608
SEM (df=56)		0.130	0.126	0.0044	0.0209	0.0072
P value						
Source		< 0.001	<0.001	<0.001	0.278	<0.001
Tannase		< 0.001	<0.001	<0.001	<0.001	0.002
Diet x Tannase interactions		0.941	0.917	0.645	0.949	0.260

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; NR, coefficient of total tract nitrogen retention; FD, coefficient of total tract fat digestibility; DMR, coefficient of total tract dry matter retention; Each mean represents values from nine replicate pens of two birds each; Dietary NR, FD and DMR were determined between 17 and 21 days of age; SEM, Standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$. ^{a,b,c}Values within a column with different superscripts differ significantly.

6. 3. 3. Effect of the experimental diets on the gastrointestinal tract components of broilers

Feeding the experimental diets did not influence ($P>0.05$) the weight and relative weight of the small intestine of the birds (Tables 6. 4 and 6. 5). Dietary inclusion of Maris Bead and Sultan beans increased ($P<0.001$) the weight and relative weight of the PG (Tables 6. 4 and 6.5). Also, dietary inclusion of beans increased PG% compared to control fed birds. Birds fed the control diet had smaller ($P<0.05$) pancreas compared to the rest, and lower ($P<0.05$) total GIT% than those fed Sultan. Overall, the enzyme mixture supplementation reduced ($P<0.05$) the weight and relative weights of the total GIT, PG and the pancreas, but did not influence ($P>0.05$) those of the small intestine (Tables 6. 4 and 6.5). A contrast

test showed that compared to the control, birds fed bean containing diets had increased pancreas (P<0.001), PG (P<0.001), and total GIT (P<0.05), although none of the treatments changed weight of the small intestine (P>0.05). There were no significant (P>0.05) diet x enzyme interactions except for PG weight (g and %) (P<0.05).

Table 6. 4. The effect of experimental diets on the gastrointestinal tract components of broiler chickens

Treatment factor		Total GIT (g)	PG (g)	Pancreas (g)	SI (g)
Diet					
Bean (Maris Bead)		70.83	25.04[b]	3.82[b]	41.96
Bean (Wizard)		68.17	22.73[a]	3.71[b]	41.73
Bean (Sultan)		70.11	24.34[b]	3.86[b]	41.91
Soybean meal (Control)		69.93	21.94[a]	3.35[a]	44.64
SEM (df=56)		1.429	0.506	0.108	1.142
Tannase					
No		71.23	24.33	3.82	43.09
Yes		68.29	22.70	3.56	42.03
SEM (df=56)		1.011	0.357	0.0764	0.807
Field bean cultivar or control diet	Enzyme				
Maris Bead	No	26.72	72.51	4.08	41.71
Maris Bead	Yes	23.36	69.14	3.57	42.22
Wizard	No	23.84	70.62	3.84	42.94
Wizard	Yes	21.63	65.72	3.58	40.52
Sultan	No	25.16	72.68	4.00	43.51
Sultan	Yes	23.53	67.55	3.72	40.31
Soybean meal	No	21.58	69.10	3.33	44.18
Soybean meal	Yes	22.30	70.75	3.36	45.09
SEM (df=56)		2.021	0.715	0.153	1.614
P value					
Source		0.604	< 0.001	0.005	0.230
Tannase		0.045	0.002	0.021	0.360
Diet x Tannase interactions		0.311	0.043	0.384	0.490

GIT, gastrointestinal tract weight (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard weight; SI, small intestine weight (including duodenum, jejunum and ileum); Each value represents mean of nine replicate pens; Gastrointestinal tract components were measured at 21 days of age using heavier bird in each pen; SEM, Standard error of the mean; There is a statistically significant difference between treatments when P≤0.05. [a,b]Values within a column with different superscripts differ significantly.

Table 6. 5. The effect of experimental diets on the gastrointestinal tract components of broiler chickens (data presented as a percentage of the body weight)

Treatment factor		Total GIT%	PG%	Pancreas%	SI%
Diet					
Bean (Maris Bead)		7.75[ab]	2.75[c]	0.42[b]	4.59
Bean (Wizard)		7.65[ab]	2.55[b]	0.42[b]	4.68
Bean (Sultan)		7.96[b]	2.76[c]	0.44[b]	4.76
Soybean meal (Control)		7.41[a]	2.33[a]	0.36[a]	4.72
SEM (df=56)		0.126	0.058	0.01174	0.107
Tannase					
No		7.87	2.69	0.42	4.76
Yes		7.51	2.50	0.39	4.62
SEM (df=56)		0.089	0.041	0.008	0.076
Field bean cultivar or control diet	Enzyme				
Maris Bead	No	7.92	2.93	0.45	4.55
Maris Bead	Yes	7.58	2.56	0.39	4.63
Wizard	No	7.87	2.66	0.43	4.78
Wizard	Yes	7.42	2.45	0.40	4.57
Sultan	No	8.20	2.84	0.45	4.91
Sultan	Yes	7.71	2.69	0.43	4.60
Soybean meal	No	7.48	2.34	0.36	4.78
Soybean meal	Yes	7.34	2.32	0.35	4.66
SEM (df=56)		0.179	0.082	0.0166	0.151
P value					
Source		0.025	< 0.001	< 0.001	0.713
Tannase		0.007	0.002	0.018	0.192
Diet x Tannase interactions		0.764	0.194	0.612	0.617

GIT%, Gastrointestinal tract (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG%, Proventriculus and gizzard; SI %, Small intestine (including duodenum, jejunum and ileum); Each mean represents values from nine replicate pens; Gastrointestinal tract components were measured at 21 days of age using heavier bird in each pen; SEM, Standard error of the mean; There is a statistically significant difference between treatments when $P \leq 0.05$. [a,b,c]Values within a column with different superscripts differ significantly.

6. 3. 4. Effect of the experimental diets on ileal digesta viscosity and mucin losses (as sialic acid secretion) in the excreta of broilers

Dietary enzyme mixture reduced (*P*<0.001) viscosity by 46.5% (Table 6. 6). Feeding diets containing Sultan (high in tannin contents), reduced (*P*=0.005) the concentration of SA in excreta compared to the rest of the diets. Also, feeding all bean-containing diets resulted in lower (*P*<0.001) total SA secretion than the control diet. Feeding enzyme however, did not influence (*P*>0.05) SA concentration, but reduced (*P*<0.001) total SA secretion by 9.4%. Compared to the mean of the bean diets, feeding the control diet increased (*P*<0.05) ileal digesta viscosity (8.31 *versus* 6.78 cP), and total SA secretion (*P*<0.001) (329 *versus* 257). No significant (*P*>0.05) diet x enzymes interaction were observed.

Table 6. 6. Ileal digesta viscosity (cPa) and mucin losses (SA) responses to the experimental diets

Treatment factor		cPa	SAc (µg/g DM)	SAt (µg)
Diet				
Bean (Maris Bead)		7.12	1.01[b]	256[a]
Bean (Wizard)		6.82	1.03[b]	255[a]
Bean (Sultan)		6.40	0.94[a]	259[a]
Soybean meal (Control)		8.31	1.03[b]	329[b]
SEM (df=56)		0.548	0.020	7.8
Tannase				
No		9.33	1.01	288
Yes		4.99	1.00	261
SEM (df=56)		0.387	0.014	5.5
Field bean cultivar or control diet	Enzyme			
Maris Bead	No	9.53	1.04	275
Maris Bead	Yes	4.70	0.98	237
Wizard	No	9.07	1.03	272
Wizard	Yes	4.56	1.03	237
Sultan	No	8.44	0.96	277
Sultan	Yes	4.36	0.92	242
Soybean meal	No	10.30	1.01	329
Soybean meal	Yes	6.32	1.06	328
SEM (df=56)		0.775	0.028	11.0
P value				
Source		0.096	0.005	<0.001
Tannase		<0.001	0.628	<0.001
Diet x Tannase interactions		0.940	0.193	0.293

cPa, dynamic ileal digesta viscosity; SAc, concentration of mucin losses as sialic acid in excreta; SAt, total excreted mucin losses as sialic acid over 96 hours (from 17 to 21days of age); Each mean represents values from nine replicate pens of two birds each; Viscosity of the supernatant (in centipoise (cPa) units) was determined at 21 days of age; SEM, Standard error of the mean; There is a statistically significant difference between treatments when *P*≤0.05.[a,b]Values within a column with different superscripts differ significantly.

6. 3. 5. Effect of the experimental diets on ileal villus morphometry of broilers

The results on jejunal histomorphological parameters are presented in Table 6. 7. Feeding the control diet increased (*P*=0.038) the muscle thickness of the jejunum compared to feeding Maris Bead and Sultan bean-containing diets. The enzyme mixture supplementation only tended to (*P*=0.061) reduce the muscle thickness of the wall of the jejunum among the measured jejunal histomorphological parameters. Villus height and width were not affected (*P*>0.05) by the diets and enzyme supplementation. An orthogonal comparison contrast test showed that birds fed bean containing diets had decreased (*P*<0.001) jejunal crypt depth compared to the control fed birds (216 *versus* 240 nm) and no diet x enzyme interactions were observed (*P*>0.05).

Table 6. 7. Jejunum histomorphological variables (µm) responses to the experimental diets

Treatment factor		Muscle thickness	Crypt depth	Villus height	Villus width
Diet					
Bean (Maris Bead)		181[a]	217	1045	185
Bean (Wizard)		197[ab]	210	978	170
Bean (Sultan)		180[a]	222	999	196
Soybean meal (Control)		201[b]	240	1025	185
SEM(df=56)		6.3	8.3	33.8	10.7
Tannase					
No		196	222	1015	180
Yes		184	222	1008	188
SEM (df=56)		4.4	5.9	23.9	7.5
Field bean cultivar or control diet	Enzyme				
Maris Bead	No	193	214	1089	172
Maris Bead	Yes	169	219	1000	198
Wizard	No	205	211	953	168
Wizard	Yes	189	208	1003	171
Sultan	No	180	214	980	198
Sultan	Yes	180	230	1018	193
Soybean meal	No	204	249	1036	180
Soybean meal	Yes	197	232	1013	190
SEM (df=56)		8.8	11.8	47.8	15.1
P value					
Source		0.038	0.072	0.532	0.403
Tannase		0.061	0.979	0.856	0.435
Diet x Tannase interactions		0.525	0.573	0.447	0.765

Each value represents mean of nine replicate pens of twenty-five villus per replicate. Villus measurements were determined at 21 days of age using lighter bird in each pen; SEM, Standard error of the mean; There is a statistically significant difference between treatments when *P*≤0.05. [a,b]Values within a column with different superscripts differ significantly.

6. 4. Discussion

The purpose of the experiment reported in this chapter was to determine whether tannase-containing enzyme could be used to improve available energy and nutrient utilisation in field bean containing diets when fed to growing broiler chickens. It was important to evaluate exogenous tannase efficiency using different field bean cultivar samples because of the large variation in the agronomic production and chemical composition of beans available to the animal feed industry.

The sample of bean cultivar Sultan had higher tannin and NSP contents, followed by Wizard and Maris Bead samples. Tannins are hydro soluble and high molecular weight polyphenolic compounds. Tannins have the ability to precipitate macromolecules (such as proteins, cellulose, starch, etc.) and minerals by forming strong complexes (Lekha and Lonsane, 1997). Compared to Maris Bead and Wizard, Sultan also had a lower ME, NR and DMR most probably due to its higher tannin and soluble NSP contents. In addition, Sultan had a lower CP content. The lower ME and CP content of these diets may have directly affected growth performance. Also, in the study reported in chapter three, lower ME, nutrient utilisation and feed efficiency were observed with Sultan-containing diets than those found with Maris Bead and Wizard field bean containing diets. The slightly higher ranges of differences in ME, nutrient utilisation and feed efficiency between these field bean cultivars in this study than those found between them in the previous study may be stemmed from using a higher rate of the beans (300 g/kg diet) in the current study than that in the other study (200 g/kg diet), in addition to the variation in the composition of the balancer feeds used in both studies.

Reduced mucin losses (measured as SA) in birds fed the cultivar Sultan, may be associated with a reduced number of GIT microflora (Pirgozliev *et al.,* 2008). Redondo *et al.* (2014) also reported reduced bacterial number in excreta when birds were fed tannin containing diets. However, the birds in this study were not under specific microbial challenge, so gut health benefits from dietary tannin contents were not expected.

Tannins can form complexes with proteins and inhibit enzymes, thus tannins may stimulate pancreatic secretion in a manner analogous to that of proteinase inhibitors from legume seeds (Griffiths, 1980). This suggests an explanation for the increased pancreas size in birds fed field bean containing diets compared to the control fed birds in this study. This is in agreement with findings presented in chapter four and previous reports that also found an enlarged pancreas in broilers fed high-tannin diets (Kubena *et al.,* 1983; Ahmed *et al.,* 1991). Thus suggesting that the increase in pancreas weight of birds fed field beans might have been related to higher dietary tannin contents.

The multi enzyme preparation used in this study had not only tannase, but also xylanase, amylase, pectinase and galactosidase activities. The novel aspect of this study was to study the effect of the tannase in diets that varied in tannin contents. It was expected that the tannase enzyme would have little effect on the control diet as it was formulated to

contain no tannins. The other three diets had different tannin contents, thus a different response to tannase was also expected. The previous experiment demonstrated that tannase was effective in improving availability of energy, all nutrients and the feed efficiency for broilers fed a diet containing a high tannin field bean sample. No enzyme by diet interaction was observed in the present study, thus the multi enzyme preparation improved the feeding value of all diets with the same magnitude. Therefore, the potential for tannase alone to improve the feeding value of the diets was not dependent upon the tannin content and the other enzyme activities, such pectinase and xylanase may have been more important.

The most noticeable response to dietary enzyme mixture was in reducing digesta viscosity by 46.5%. High digesta viscosity is usually associated with high content of dietary water-soluble NSPs (Choct and Annison, 1992; Bedford, 2006). These NSPs have a significant capacity to attract and hold water and could directly interact with water molecules to form a large network or mesh-like structure, thereby increasing the viscosity of digesta. Pectinase, tannase and xylanase are known to have the ability to degrade NSPs in plants (Zyla *et al.,* 2000; García-Conesa *et al.,* 2001; Bedford, 2006), thus explaining the observed reduction in ileal digesta viscosity. However, supplementation of xylanase (2000 units/kg diet) resulted in an improvement in only AME in the experiment reported in chapter four. Xylanase though, may have been involved in reducing digesta viscosity as well as hydrolysing cell wall material and therefore releasing the previously trapped nutrients and increasing nutritive value in this study. This is probably because of existing higher xylanase activity (6100 units/kg diet) in the enzyme mixture used in this study than that previously mentioned, in addition to difference in their sources. The detrimental impact of high intestinal viscosity on dietary nutrient digestibility and absorption has been well documented (Choct and Annison, 1992; Bedford, 2006). Viscous properties have adverse effects on the diffusion and convective transport of pancreatic enzymes, substrates and the end products of the digestion process, thus dietary energy and nutrient utilisation (Isaksson *et al.,* 1982; Johnson *et al.,* 1984). Also, a negative impact of cell wall material (Longstaff and McNab, 1991a, b; Choct *et al.,* 2006; Nalle *et al.,* 2010b) and a positive impact of cell wall degrading enzymes (such as xylanase produced by *Aspergillus niger*) on the nutritional quality of legume seeds for broilers have been observed (Wiryawan *et al.,* 1995, 1997; Cowieson *et al.,* 2003). Cell wall NSPs prevent accessibility of enzymes to the encapsulated nutrients by cell wall materials (Bedford, 2002; Fontes *et al.,* 2004; Nian *et al.,* 2011) and xylanase hydrolyses these materials and allows the nutrients to be accesed by digestive enzymes (Adeola and Bedford, 2004; Gao *et al.,* 2008; Nian *et al.,* 2011), thus explaining why xylanase in this enzyme mixture may also have been involved in the observed improvements in this feeding experiment. An increase in intestinal viscosity associated with enhanced bacterial fermentation can also depress fat digestion (Dänicke *et al.,* 1999).

In agreement with the observations reported in chapter four, variation in field bean cultivar did not result in significant differences in relative weights of the GIT segments. The enzyme mixture supplementation improved feed efficiency by 3.5%, an increase that is close to the 2.6% found from the previous study in 21 day-old broilers fed field beans containing diet supplemented with the similar enzyme preparation.

The weight of the pancreas as a percentage of BW decreased with the enzyme mixture supplementation by 7.1%. This decrease is similar to the 6.4% found from the previous experiment (reported in Chapter 5) for broilers of a similar age, when fed a diet containing 300 g/kg of Sultan field beans, supplemented with the same dose of the same enzyme product. Wu *et al.* (2004) also reported a reduced weight of the pancreas by 10% when feeding 1000 units of xylanase/kg diet. In addition, Gracia *et al.* (2003) found a reduced relative weight of pancreas by 17% after adding 1720 units of α-amylase/kg diet. This indicates that secretion of pancreatic enzymes may be affected by the concentration of enzymes and substrates or products of their hydrolysis in the lumen of the small intestine following a physiological feedback mechanism (Kubena *et al.,* 1983). Tannins are able to bind to enzymes, reducing their bioavailability (Singh, 1984), thus the destruction of tannins by tannase and NSPs by carbohydrases may reduce the secretion of pancreatic enzymes. Mahagna *et al.* (1995) also reported that secretion of pancreatic amylase and proteases was reduced when chicks were fed diets supplemented with amylase and protease. The combination of fibre degrading enzymes used in this study may also have improved the availability of substrates trapped by fibres via disrupting the cell wall matrix (Parkkonen *et al.,* 1997) further reducing the need of pancreatic enzymes.

The weight of the total GIT as a percentage of BW decreased with the studied enzyme mixture supplementation by 4.6%, which is similar to the 4.5% and slightly lower than the 6.3% found by Gracia *et al.* (2003) and Wu *et al.* (2004), respectively, when feeding α-amylase or a mixture of phytase and xylanase to broilers. The weight of the PG, as a percentage of the body weight, was particularly affected and decreased by 7.1%, a decrease that is similar to the 6.1% reported in the study shown in the previous chapter when fed the same enzyme to broilers of a similar age. Wu *et al.* (2004) also reported a reduced weight of the PG (as a percentage of the body weight) by 7.4% when feeding a mixture of phytase and xylanase to broilers. A similar trend was observed by Gracia *et al.* (2003) after feeding α-amylase to broilers at a similar age. The reduction in total GIT in birds given enzyme mixture containing diets paralleled the reduction in digesta viscosity and intestinal muscle thickness and the improvement in metabolisable energy, nutrient utilisation and feed efficiency are in agreement with observations made in the earlier study (displayed in chapter five). It has been found that reduced digesta viscosity increases the mobility and decreases the transit time of digesta through the GIT of birds, consequently reducing the work of digestion, as well as increasing the utilisation of dietary nutrients (Farrel *et al.,* 1999). In general, if the efficiency of digestion is consistently suboptimal,

whether due to ingredient quality, microbial interaction of ANFs, the GIT responds by increasing in both size (surface area) and digestive enzyme output (Bedford, 2006). Birds fed multi enzyme mixture also secreted less mucin, thus supporting the view that the reduction in GIT weight in this experiment might have been related to enhanced efficiency of digestion. The decrease in crypt depth was inline with the reduction in total mucin losses in excreta of birds fed bean-containing diets comparing to control. Jejunal morphometry is not always the key factor associated with better function and production in poultry (Wu *et al.,* 2004; Pirgozliev *et al.,* 2010), thus the lack of correlation with productive performance is not surprising.

6. 5. Conclusion

It can be concluded that the feeding value of field beans with different tannin contents may vary when fed to broilers. The results from this study show that a commercial enzyme preparation containing tannase, pectinase and xylanase activities proved to be highly effective at improving the feeding value of diets for broiler chickens. The beneficial effects of the addition of the enzyme mixture to poultry diets seems to be mediated through reduced digesta viscosity and improved energy and nutrient availability. Since the results in this study were based on raw field bean-containing diets, examination of the effects of the enzyme mixture on previously heat treated field beans may be warrented.

CHAPTER 7: NUTRITIONAL VALUE OF RAW AND MICRONISED FIELD BEANS (*Vicia faba* L. var. *minor*) WITH AND WITHOUT ENZYME SUPPLEMENTATION CONTAINING TANNASE, PECTINASE AND XYLANASE ACTIVITIES FOR GROWING CHICKENS

7. 1. Introduction

In the previous study (reported in Chapter 6) it was found that the commercial enzyme preparation containing tannase, pectinase and xylanase activities improved the feeding value of all diets regardless of their tannin contents when fed to broiler chickens. However, there is a lack of information on the effect of micronising on the feeding value of field beans with different tannin contents. There is also a lack of knowledge on the interaction of a commercial tannase preparation and heat treatment. Therefore, the main objective of this experiment was to study dietary energy and nutrient availability, growth performance, gastrointestinal tract components, tannin digestibility and mucin losses (as sialic acid secretion in excreta) of 6 to 16 day-old broiler chickens, when fed on diets containing two different tannin-containing raw and micronised field bean cultivar samples, with and without inclusion of exogenous enzyme preparation containing tannase, pectinase and xylanase activities.

7. 2. Materials and methods

7. 2. 1. Field bean samples

This report focuses on the nutritional value for broilers of two UK grown field bean samples that were fed either as raw or as micronised to broiler chickens. The two field bean samples used in the study were Maris Bead (spring cultivar) and Sultan (winter cultivar). Both cultivar samples were produced in the UK during 2013 harvest year, and were stored in porous synthetic bags at ambient air temperatures in a dark, dry store. The samples were chosen because of their different tannin contents, although there were differences in their proximate composition. The stored field bean samples did not experience any freezing temperatures during this period. The bean samples were milled through a 4 mm screen. Each sample was then split into two batches, one of which was micronised (130ºC, 90 seconds, 2 microns wave length; Heraeus Noblelight GmbH, Germany). Freshly milled field bean samples as raw or micronised were utilised for the feeding study to avoid spoilage. Representative samples of freshly ground raw and micronised field bean samples, the balancer diet and other feed ingredients were used for laboratory analysis.

7. 2. 2. Dietary treatments

A control diet was prepared that had the main ingredients of 400.0 g/kg wheat and 127.0 g/kg full fat SBM, and contained 221 g/kg CP and 12.83 MJ/kg ME in accordance with the breeder's recommendation (Aviagen Ltd., Edinburgh, UK) (Table 7. 1). To reduce nutrient density the control diet also contained 119.1 g/kg washed sand.

Table 7. 1. Ingredient composition (g/kg, as-fed) of the experimental broiler chicken diet formulations

Ingredient	Control	Maris beads	Sultan
Wheat	400.0	404.2	404.2
Maris bead	-	300.0	-
Sultan	-	-	300.0
SBM (CP=48%)	190.4	27.0	27.0
Full fat soybean meal	127.0	127.5	127.5
Maize gluten meal	35.0	35.0	35.0
Washed sand	119.1	-	-
Soya oil	82.5	65.0	65.0
L-Lysine-HCL	6.0	2.3	2.3
Methionine	6.8	5.8	5.8
Threonine	2.4	2.4	2.4
Monocalcium phosphate	10.0	10.0	10.0
Limestone	14.0	14.0	14.0
Salt	2.8	2.8	2.8
Vitamin/mineral premix*	4.0	4.0	4.0
Total	1000	1000	1000
Analysed values (as-fed)			
DM	855	877	876
GE (MJ/kg)	16.21	17.57	17.52
CP	197	198	183
Fat	112	95	95
Total phenols[a]	1.31	2.76 (2.66)	3.78 (3.63)
Tannins[a]	0.45	1.98 (1.77)	2.54 (2.42)
Condensed tannins[b]	0.00	1.15 (0.95)	1.86 (1.54)
Calculated values			
ME (MJ/kg)	12.83	13.12	12.65
Protein	221	217	201
Fat	113	97	97
Calsium (g/kg)	7.9	8.1	8.2
Available phosphorus (g/kg)	4.4	4.4	4.4
Total lysine (g/kg)	15.1	12.4	11.8
Total methionine + cysteine (g/kg)	13.5	8.6	8.4

Values in brackets are valid for diets with micronised bean samples; *Vitamin and mineral premix provided per kg diet: 30 mg niacin, 25 mg α-tocopherol, 8 mg pantotenic acid, 5 mg riboflavin, 2.16 mg retinol, 1.5 mg pyridoxine, 1.5 mg menadione, 1.5 mg thiamine, 0.5 mg folic acid, 75 *μ*g cholecalciferol, 60 *μ*g biotin, 10 *μ*g cyanocobalamin, 80 mg manganese, 80 mg iron, 80 mg zinc, 10 mg copper, 0.8 mg iodine and 0.3 mg selenium; Diets were not supplemented with coccidiostat; [a] As tannic acid equivalents; [b] As leucocyanidin equivalents; The contents of total phenols, tannins and condensed tannins in the ingredients of diets containing field beans was 1.42 g/kg, 0.60 g/kg and 0.00 g/kg, respectively.

After adjusting the dry matter of the micronised field bean samples, four more diets containing 300 g/kg of experimental field bean cultivar samples, two untreated and two micronised, in replacement for SBM and sand were mixed in order to have ME and CP in a range similar to those of the control diet (Table 7. 1). Each diet was then split into two batches, one of which was supplemented with an enzyme mixture (Kerry Ingredients and Flavours, Osberstown, Naas, Co. Kildare, Ireland) resulting in ten experimental diets in total. The determined enzyme activities of the enzyme mixture were; tannase 3400 units/kg diet, pectinase 6220 units/kg diet; xylanase 6100 units/kg diet, and there were some additional amylase and α-galactosidase activities. The enzyme preparation was produced by *Aspergillus niger* in a submerged fermentation methodology. The enzyme was in a liquid form and the reported enzyme activities were obtained after spraying 17 ml/kg on top of the diets. The DM content of non-supplemented diets was adjusted by spraying 17 ml of water per kg of diet. Each diet was thoroughly mixed in a horizontal mixer and fed as mash. Diets were free of coccidiostat, antimicrobial growth promoters, prophylactic and other similar additives.

7. 2. 3. Animal husbandry, experimental design, determination of dietary metabolisable energy, nutrient utilisation, tannin degradability, mucin losses and broiler growth performance

The experiment was approved by Research Ethics Committee at Harper Adams University.

One hundred and sixty day-old male Ross 308 broiler chicks were obtained from a commercial hatchery. During the pre-study period, from day old to 6 days of age, the birds were reared in a single floor pen and fed a proprietary wheat-based diet without coccidiostats or antimicrobial growth promoters, or other similar additives. At the beginning of the study, at 7 days of age, 140 chicks were allocated to 70 small pens with 0.16 m^2 solid floors area, two birds in each pen. Birds had free access to feed and water over the experimental period. Each diet was offered to birds in 7 pens in a randomised block design. Information on growth performance was obtained from 7 to 16 days of age. The temperature was kept at 29°C at 7 days of age and was gradually reduced to 22°C at the end of the 10 day feeding period (16 days of age). The light regimen was 18 hours light and 6 hours dark. At 12 days of age, the solid floor of each pen was replaced with a wire mesh and excreta samples were quantitatively collected for four consecutive days from each pen and immediately dried at 60°C until a constant weight. The dried excreta were then milled (passing a 0.8 mm pore diameter sieve) after leaving at room temperature over three nights and weighing. The feed intake was measured for the same time as well. The birds were weighed at the beginning (day 6) and the end (day 16) of the study, and the diets were weighed at the beginning (day 6), day 12 and the end (day 16) of the study on a per pen basis. Daily feed intake (FI), daily weight gain (WG) and feed

conversion ratio (FCR) were determined. The GE, DM, nitrogen and fat of each dried excreta sample and the experimental diets were determined as described earlier (see Section 2. 2. 2. 1). The AME and AMEn of the diets were calculated as described by Hill and Anderson (1958). The coefficients of total tract fat (FD) digestibility, dry matter (DMR) and nitrogen (NR) retention were determined as the difference between intake and excretion of the nutrient divided by its respective intake (see Section 3. 2. 3).

The total phenols, non-tannin phenols and total tannins (all as tannic acid equivalents) in the representative samples of excreta, as well as freshly milled raw and micronised studied field bean cultivars, the control diet and other feed ingredients were determined by applying the procedure suggested by Makkar *et al.* (1993). Condensed tannins, as leucocyanidin equivalents, in the same samples were determined by using the assay described by Porter *et al*. (1985) as described before (see Section 2. 2. 2. 7).

The degradation in the GIT of tannins was described as tannin degradability (TD), when tannins were presented as tannic acid equivalents, and as condensed tannin degradability (CTD), when tannins were presented as leucocyanidin equivalents. The TD and CTD were determined as the difference between the intake and the excretion of the anti-nutrient, divided by its respective intake. The mucin losses in excreta were measured using the concentration of the sialic acid (SA) as a marker, following the periodate-resorcinol method (Jourdian *et al.,* 1971), as described in the previous experiment (see Section 6. 2. 3).

7. 2. 4. Gastrointestinal tract components

At the end of the experiment, at 16 days of age, all birds were killed by cervical dislocation and weighed. The empty and relative weights of the GIT segments from proventriculus to caeca of the heavier bird in each pen were determined according to the procedure used by Amerah and Ravindran (2008).

7. 2. 5. Statistical analysis

Data was analysed statistically using the GenStat statistical software package (Genstat 15th release 3.22 for Windows; IACR, Rothamstead, Hertfordshire, UK). The experiment was arranged as a randomised block analysis of variance with 10 treatments each with 7 replicates. The treatments were arranged 2 x 2 x 2 factorial with a further two specific orthogonal contrasts for the control diets. The 2 x 2 x 2 factorial arrangement had field bean cultivar (Maris Bead or Sultan), enzyme (with and without tannase) and micronising (with and without). The first specific orthogonal contrast was Control 1 (no enzyme) *versus* Control 2 (with enzyme), and the second contrast was mean of all bean diets *versus* mean of the two control diets. In all instances, differences were reported as significant at $P \leq 0.05$. Tendencies towards significance ($P \leq 0.10$) were also reported.

7. 3. Results

7. 3. 1. Effect of micronising on chemical composition of the experimental field bean cultivar samples

Micronising slightly reduced the total phenol, tannin (both as tannic acid equivalents) and condensed tannin (as leucocyanidin equivalents) contents in both of the field bean cultivars (Table 7. 2).

Table 7. 2. Chemical composition of the experimental field bean cultivar samples

Ingredient	Field bean cultivar			
	Maris Bead		Sultan	
	Raw	Micronised	Raw	Micronised
Dry matter (g/kg)	854	883	851	887
Total phenols (g/kg DM)[a]	6.9	6.3	10.9	9.9
Tannins (g/kg DM)[a]	6.1	5.1	8.3	7.5
Condensed tannins (g/kg DM)[b]	4.5	3.6	7.3	5.8

[a] As tannic acid equivalent; [b] As leucocyanidin equivalent; all analyses were analysed in triplicate.

7. 3. 2. Broiler growth performance, available energy, nutrient availability and tannin degradability coefficients of the experimental diets

Results on dietary available energy, nutrient utilisation and tannin degradability coefficients, and growth performance of broilers are summarised in Table 7. 3. The birds fed field bean diets had a lower (P<0.001) daily feed intake and weight gain, but higher (P<0.001) FCR than the birds fed the control diets. Bean based diets had lower NR (P<0.001), and FD (P=0.009), but a higher determined AME (P=0.002), AMEn and DMR (P<0.001) compared to the control diet.

The enzyme supplemented diets had better (P<0.05) FCR compared to un-supplemented diets. For some reasons non enzyme supplemented control diet had higher (P=0.004) NR than the supplemented diets, but no difference (P>0.05) in FD was observed. Overall, the multi enzyme supplemented diets had higher AME, AMEn, NR, DMR (P<0.001) and FD (P=0.002), than un-supplemented diets.

Compared to Sultan, feeding Maris Bead resulted in a better (P<0.001) FCR, a higher (P<0.001) WG and DMR, and a higher (P<0.05) determined metabolisable energy. There was a three way interaction (bean x enzyme x micronising; P=0.033) for FCR as diet containing non-micronised Maris Bead with the enzyme mixture had a lower FCR, although the response of the rest of the diets was inconsistent.

There was a bean by micronising interaction (P=0.043) in TD, as the TD for Sultan-containing diet was reduced with micronising although no reduction (P>0.05) was observed for Maris Bead based-diet. Maris Bead based diets had lower CTD (P<0.001),

than Sultan based diets. Micronised bean-containing diets had lower CTD (P<0.001), than non-micronised bean-containing diets.

7. 3. 3. Effect of the experimental diets on mucin losses (as sialic acid secretion in excreta) and gastrointestinal tract components of broiler chickens

The results on mucin losses secretion, measured as SA, in excreta and gastrointestinal tract comonents responses to the experimental diets are displayed in Table 7. 4. The SA concentration was reduced in bean-containing diets (P=0.042), Sultan based diets (P=0.009) and in non-micronised bean-based diets (P=0.034), compared to controls, Maris Bead and micronised bean-containing diets, respectively.

The weight of the total GIT was reduced by feeding Sultan compared to Maris Bead containing diets (P=0.018) and the enzyme mixture supplemented compared to none supplemented diets (P=0.020). When expressed as a percent from the body weight the total GIT was increased by feeding bean containing diets compared to controls (P<0.001), Sultan compared to Maris Bead based diet (P=0.011) and non-supplemented compared to those with the enzyme (P=0.003).

The weight of the PG was increased by feeding bean containing diets compared to controls (P=0.010) and when comparing enzyme free to the enzyme supplemented diets (P=0.003). Similarly, the PG% was increased by feeding bean containing diets compared to controls (P<0.001), Sultan compared to Maris Bead based diet (P=0.031) and non-supplemented compared to the enzyme supplemented diets (P=0.001).

The weight of the SI was reduced by feeding bean containing compared to control diets (P<0.001) and Sultan compared to Maris Bead containing diet (P=0.024). For SI%, only tendencies were observed.

The weight of the pancreas was not affected (P>0.05) by any of the treatments. However, the pancreas% was increased (P<0.001) by feeding bean containing diets compared to controls.

Table 7. 3. Broiler growth performance, available energy and nutrients, and tannin digestibility coefficients of the experimental diets

Treatment factor	FI (DM g/b/d)	WG (g/b/d)	FCR	AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR	TD	CTD
1 Control	39.7	28.9	1.377	13.55	12.66	0.678	0.758	0.611	0.362	0.483
2 Maris Bead raw	40.7	31.0	1.314	13.46	12.67	0.653	0.737	0.614	0.281	0.483
3 Sultan raw	36.8	26. 6	1.386	13.73	12.95	0.629	0.659	0.642	0.351	0.504
4 Maris Bead micronised	34.2	26.4	1.298	14.26	13.45	0.652	0.727	0.662	0.330	0.499
5 Sultan micronised	37.0	26.9	1.377	13.71	12.95	0.624	0.708	0.642	0.169	0.363
6 Control + Enzyme	35.4	26.4	1.343	14.28	13.49	0.642	0.718	0.666	0.243	0.395
7 Maris Bead raw + Enzyme	34.8	23.7	1.471	13.41	12.68	0.635	0.661	0.625	0.301	0.532
8 Sultan raw + Enzyme	35.3	23.8	1.492	13.89	13.16	0.643	0.712	0.647	0.393	0.577
9 Maris Bead micronised + Enzyme	35.1	23.5	1.495	13.36	12.65	0.622	0.682	0.609	0.348	0.485
10 Sultan micronised + Enzyme	33.8	23.6	1.440	14.15	13.43	0.643	0.748	0.652	0.360	0.511
SEM (n=7)	1.35	1.10	0.0209	0.136	0.131	0.0058	0.0213	0.0060	0.0484	0.0302
Specific orthogonal contrasts										
Beans x Enzyme x Micronising										
Bean cultivar										
Maris Bead (n=28)	35.8	26.6	1.351	13.99	13.21	0.637	0.703	0.653	0.273	0.440
Sultan (n=28)	34.8	23.7	1.474	13.70	12.98	0.636	0.701	0.633	0.350	0.527
Enzyme										
No enzyme (n=28)	35.9	25.2	1.432	13.55	12.81	0.627	0.677	0.629	0.292	0.471
Enzyme (n=28)	34.7	25.1	1.393	14.15	13.38	0.645	0.726	0.657	0.331	0.496
Micronising										
No micronised (n=28)	35.3	25.1	1.412	13.82	13.06	0.640	0.690	0.644	0.344	0.528
Micronised (n=28)	35.3	25.1	1.414	13.88	13.13	0.633	0.714	0.642	0.280	0.439
SEM (n=28)	0.675	0.548	0.0105	0.068	0.066	0.0029	0.0106	0.0030	0.0242	0.0151
Beans *versus* Controls										
Beans (n=56)	35.3	25.1	1.413	13.85	13.10	0.636	0.702	0.643	0.312	0.483
Control (n=14)	40.2	30.0	1.345	13.51	12.66	0.665	0.748	0.612	0.322	0.483
SEM (min–max replicate)*	0.96-0.48	0.78-0.39	0.0148-0.0074	0.096-0.048	0.093-0.046	0.0041-0.0021	0.0150-0.0750	0.0043-0.0021	0.0342-0.0171	0.0214-0.0107
Probabilities of differences										
Bean cultivar (B)	0.261	<.001	<.001	0.004	0.017	0.814	0.880	<.001	0.028	<.001
Enzyme (E)	0.209	0.887	0.011	<.001	<.001	<.001	0.002	<.001	0.258	0.260
Micronised (M)	0.966	0.989	0.902	0.572	0.455	0.108	0.113	0.671	0.067	<.001
B x E	0.383	0.773	0.147	0.661	0.557	0.472	0.528	0.213	0.703	0.605
B x M	0.483	0.787	0.278	0.609	0.607	0.943	0.803	0.346	0.043	0.128
E x M	0.833	0.909	0.728	0.347	0.356	0.598	0.469	0.137	0.913	0.840
B x E x M	0.463	0.952	0.033	0.460	0.460	0.283	0.232	0.333	0.205	0.523
Probabilities of other specific contrasts										
Control 1 (n=7) *versus* Control 2 (n=7)	0.598	0.183	0.037	0.635	0.928	0.004	0.472	0.771	0.244	<0.001
Beans (n=56) *versus* Control (n=14)	<.001	<.001	<.001	0.002	<.001	<.001	0.009	<.001	0.800	0.260

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy; NR, coefficient of total tract nitrogen retention; FD, coefficient of total tract fat digestibility; DMR, coefficient of total tract dry matter retention; TD, coefficient of total tannin digestibility; CTD, coefficient of condensed tannin digestibility. Each mean represents values from seven replicate pens of two birds each; bird performance was determined from 6 to 16 days of age; dietary AME, AMEn, DMR, NR, FD, TD and CTD were determined from 12 to 16 days of age. *Notes: SEM, Standard error of the mean; There is statistically significant difference between treatments when $P \leq 0.05$.

Table 7. 4. Mucin losses (as sialic acid secretion in excreta) and responses of the gastrointestinal tract components to the experimental diets

Treatment factor	SAc (mg/g)	SAt (mg)	Total GIT (g)	Total GIT%	PG (g)	PG%	Pancreas (g)	Pancreas%	SI (g)	SI%
1 Control	1.19	0.18	36.54	8.67	13.50	3.04	1.95	0.44	21.09	5.18
2 Maris Bead raw	1.15	0.18	37.50	8.57	14.23	3.09	1.91	0.42	21.36	5.06
3 Sultan raw	1.13	0.16	38.36	9.71	16.96	4.07	2.21	0.53	19.19	5.12
4 Maris Bead micronised	1.14	0.16	36.06	9.46	14.93	3.70	2.09	0.52	19.04	5.24
5 Sultan micronised	1.19	0.17	38.04	9.85	15.97	3.91	2.18	0.53	19.89	5.42
6 Control + Enzyme	1.14	0.17	36.33	9.39	14.75	3.62	2.06	0.50	19.52	5.27
7 Maris Bead raw + Enzyme	1.06	0.16	36.75	10.17	15.98	4.18	2.10	0.55	18.67	5.44
8 Sultan raw + Enzyme	1.11	0.17	34.01	9.78	14.58	3.97	1.89	0.51	17.54	5.29
9 Maris Bead micronised + Enzyme	1.13	0.17	35.56	10.38	14.89	4.12	2.01	0.56	18.65	5.70
10 Sultan micronised + Enzyme	1.12	0.20	33.24	9.65	13.85	3.80	2.02	0.55	17.36	5.30
SEM (n=7)	0.024	0.012	1.339	0.210	0.650	0.124	0.118	0.025	0.823	0.144
Specific orthogonal contrasts										
Beans x Enzyme x Micronising										
Bean cultivar										
Maris Beads (n=28)	1.15	0.17	37.20	9.60	15.65	3.82	2.13	0.52	19.41	5.26
Sultan (n=28)	1.10	0.17	34.89	10.00	14.82	4.02	2.01	0.54	18.06	5.44
Enzyme										
No enzyme (n=28)	1.13	0.17	37.17	10.03	15.95	4.07	2.12	0.54	19.10	5.42
Enzyme (n=28)	1.13	0.18	34.91	9.57	14.53	3.77	2.02	0.52	18.37	5.28
Micronising										
No micronised (n=28)	1.11	0.16	36.29	9.78	15.61	3.98	2.07	0.53	18.61	5.27
Micronised (n=28)	1.15	0.18	35.79	9.82	14.87	3.86	2.07	0.54	18.86	5.42
SEM (n=28)	0.012	0.006	0.669	0.105	0.325	0.062	0.059	0.013	0.412	0.072
Beans *versus* Controls										
Beans (n=56)	1.13	0.17	36.04	9.80	15.24	3.92	2.07	0.53	18.73	5.35
Control (n=14)	1.17	0.18	37.02	8.62	13.86	3.07	1.93	0.43	21.22	5.12
SEM (min – max replicate)*	0.017-0.009	0.009-0.004	0.947-0.473	0.149-0.074	0.460-0.230	0.088-0.044	0.083-0.042	0.018-0.09	0.582-0.291	0.102-0.051
Probabilities of differences										
Bean cultivar (B)	0.009	0.624	0.018	0.011	0.077	0.031	0.133	0.231	0.024	0.090
Enzyme (E)	0.897	0.264	0.020	0.003	0.003	0.001	0.200	0.284	0.212	0.163
Micronised (M)	0.034	0.099	0.597	0.785	0.112	0.184	0.956	0.629	0.676	0.147
B x E	0.339	0.281	0.781	0.501	0.664	0.729	0.902	0.885	0.418	0.213
B x M	0.784	0.669	0.615	0.997	0.727	0.981	0.763	0.440	0.558	0.879
E x M	0.081	0.718	0.791	0.377	0.531	0.950	0.527	0.968	0.876	0.216
B x E x M	0.920	0.712	0.964	0.831	0.807	0.589	0.505	0.492	0.981	0.973
Probabilities of other specific contrasts										
Control 1 (n=7) *versus* Control 2 (n=7)	0.222	0.791	0.613	0.749	0.433	0.771	0.825	0.574	0.814	0.537
Beans (n=56) *versus* Control (n=14)	0.042	0.281	0.360	<.001	0.010	<.001	0.150	<.001	<.001	0.052

SAc, concentration of mucin losses as sialic acid in excreta; SAt, total excreted mucin losses as sialic acid over 96 hours (from 12 to16 days of age); GIT, gastrointestinal tract weight (including pancreas, proventriculus and gizzard, duodenum, jejunum and ileum); PG, proventriculus and gizzard weight; SI, small intestine weight (including duodenum, jejunum and ileum); GIT%, gastrointestinal tract as a proportion to the body weight; PG%, proventriculus and gizzard as a proportion to the body weight; SI%, small intestine as a proportion to the body weight; Each mean represents values from seven replicate pens; gastrointestinal tract components were measured at 16 days of age using the heavier bird in each pen; endogenous mucin losses (as sialic acid) in excreta was measured in excreta collected from 12 to 16 days of age; SEM, standard error of the means; there is statistically significant difference between treatments when $P \leq 0.05$.

7. 4. Discussion

The objective of the study reported in this chapter was to examine if heat treatment (micronising) of field beans and exogenous enzyme preparation containing tannase, pectinase and xylanase activities could be used to improve available energy and nutrient utilisation in diets for broilers. It was crucial to test these treatments employing various field bean cultivar samples, due to existing large differences in the agronomic production and chemical composition of available beans to the animal feed industry.

Tannins can form strong complexes with proteins, starch, cellulose, and minerals (Lekha and Lonsane, 1997), and can decrease the availability of dietary energy (Vilariño *et al.,* 2009), protein, AA (Woyengo and Nyachoti, 2012) and minerals (Marquardt and Ward, 1979) for broiler chickens. The sample of bean cultivar Sultan had a higher tannin content compared to Maris Bead sample. Similar to the previous study, Sultan had a lower metabolisable energy, most probably due to its higher NSP contents, than Maris Bead. In addition, Sultan had a lower CP content. The lower metabolisable energy and CP content of these diets may have directly affected growth performance. In agreement with the finding in the previous study, feeding Sultan-containing diet reduced mucin loss in excreta to a significantly lower level than that found in excreta of birds fed on Maris Bead-containing diets. However, the impact of dietary treatments on the microflora was not studied in this experiment, but reduced mucin losses in birds fed the cultivar Sultan compared to Maris Bead-based diet may have been associated with a reduction in the gut microflora (Pirgozliev *et al.,* 2008), due to higher concentration of tannins in Sultan beans. A reduction in bacterial population in excreta of birds following consumption of tannin containing diets have also been reported previously by Redondo *et al.* (2014). This experiment showed that there were no differences in nutritional value between the raw and heat treated field beans. Alonso *et al.* (2000a) demonstrated that heat treatment (extrusion) gave a two-fold reduction in condensed tannins in faba beans, while in the present study heat treatment only gave approximately 9% reduction in condensed tannins. However, there is a difference between the process of autoclaving and micronising, as extruding requires higher temperature, some water and relatively more time compared to micronising (Lashkari *et al.,* 2015).

The reduced CTD of micronised diets, and the observed interactions where micronising reduced feed efficiency and TD of Maris Bead based diet only, were not expected. Bellido *et al.* (2006) reported that micronising legumes, e.g. cowpea flour, at 130ºC changed its functional properties, including reduced foaming capacity, increase in the surface hydrophobicity and cross-linking of the protein, formation of disulphide bonds and possibly Maillard cross-links. It is possible that Sultan and Maris Bead field beans reacted differently to the heat treatment applied in this experiment.

From the study presented in chapter five, results showed that the exogenous enzyme mixture was effective in improving dietary energy and nutrient availability and the feed efficiency for broilers fed a diet containing high tannin field beans. In agreement with the findings reported in chapter six, exogenous enzyme product improved the feeding value of all diets. Apart from the interaction for FCR, no other enzymes by diet interactions were observed in the present study. This shows that the exogenous enzyme mixture improved the feeding value of all diets regardless of their tannin contents and micronising treatment with the same magnitude. This finding is supported by the lack of significant positive impact of the enzyme mixture supplementation on tannin degradability. Chamorro *et al.* (2015) found no effect of tannase supplementation on growth performance in chickens fed diets rich in polyphenols. The enzyme mixture used in the present experiment also had α-amylase, xylanase, and pectinase activities. It is possible that these enzyme activities may have been partially responsible for the observed improvements in the availability of energy and nutrients, and feed efficiency in the study.

The most noticeable response to the dietary enzyme mixture was the increase in FD (7.1%), dietary metabolisable energy (4.4%), DMR (4.5%) and dietary NR (2.9%). These results are similar to those reported in the former study (presented in chapter six) and close to those shown in chapter five when broiler chickens were fed a field bean-based diet supplemented with the same enzyme product. Although there was an increased dietary NR when the enzyme mixture was fed, NR is influenced not only by protein digestibility, but also by metabolic N excretion (Souffrant, 2001). It is generally accepted that part of the anti-nutritional effect of field beans is also mediated by its NSP constituents (Longstaff and McNab, 1991a, b; Nalle, 2009) that raise the viscosity of gut contents and may alter the microflora (Smits *et al.,* 1998; Langhout *et al.,* 1999). An increase in intestinal viscosity associated with enhanced bacterial fermentation can also depress fat digestion (Dänicke *et al.,* 1999).

The weight of total GIT as a percentage of BW decreased with the multi enzyme supplementation by 4.6%, which is similar to the reduction (4.6%) found in the previous study with the same enzyme. This reduction in the total GIT was also similar to the decrease reported by Gracia *et al.* (2003) (4.5%) and comparable to that documented by Wu *et al.* (2004) (6.3%), when feeding α-amylase or a mixture of phytase and xylanase to broilers. The weight of PG as a percentage of BW decreased by 7.4%, which is similar to the decrease (6.1 and 7.0%) reported in two previous studies (shown in chapter five and six), when fed the same enzyme to broiler chickens. Wu *et al.* (2004) also reported a reduced weight of the PG by 7.4% when feeding a mixture of phytase and xylanase to broilers. A similar trend was observed by Gracia *et al.* (2003) after feeding α-amylase to broilers at a similar age. The changes in GIT expressed as % of the weight of the birds

were similar to the absolute values. In general, if the efficiency of digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction of NSPs, the GIT responds by increasing in both size (surface area) and digestive enzyme output (Bedford, 2006).

7. 5. Conclusion

The results from this study demonstrate that there can be large differences in the nutritional value of different field bean samples that are available to the poultry feed industry. Application of heat treatment (micronising) did not improve the nutritional value of either bean sample. The addition of a commercial tannase enzyme preparation (that additionally had alpha-amylase, xylanase, and pectinase activities) proved to be highly effective in improving dietary available energy and nutrient utilisation in chickens. Further research is warranted to elucidate the effectiveness of tannase supplementation in poultry diet formulations by dose response trials with purified tannase preparations. Similarly, more research is needed on the impact of various temperatures and processing times on the feeding value of field beans for broiler chickens.

CHAPTER 8: GENERAL DISCUSSION

It is a current objective of the European Union that food security should be improved by member states (Schreuder and de Visser, 2014). The increased use of home grown protein sources, to replace the dependence on imported SBM in animal feeds, is an obvious direction that the agricultural industry should explore. Field beans are being increasingly grown in the UK (PGRO, 2015, 2016) and are now becoming available for use in animal feeds. During the past five years, the cropping area and the yield of field beans in the UK has steadily increased. Only for 2016 the field bean production increased by 42% compared to the average production for the past 5 years (578 000 vs 406 000 tonnes), also the amount of beans available as animal feed increased accordingly (PGRO 2015, 2016). Additionally, field beans are cheap (£156/tonne) comparing to SBM (£345/tonne). Currently, relatively small amounts of field beans are used in UK broiler feeds and the industry has only a limited experience of their use in practical diets.

This research programme was intended to provide information to be directly relevant to the poultry feed industry. This project has given information in three major areas. First, the animal feed industry needs to know the exact nutrient composition of the currently available cultivars and the bioavailablility of these nutrients for use when formulating economically efficient diets. In addition, the animal feed industry needs to know the variability between different field bean samples to use when they employ stochastic linear feed formulation methods. Second, the industry needs information to decide what quality measures they should apply when receiving deliveries of field beans and how to identify a nutritionally superior batch. Third, the poultry industry needs information on whether there are methods to improve, or reduce variability, in the nutritional value of field beans. The objective of this general discussion is to consider each of these three main areas and give general conclusions that are directly relevant to the broiler chicken feed industry.

8. 1. Nutrient composition and nutrient bioavailablility of field beans

Accurate information on the nutrient content and nutrient availability of field beans is crucial and enables the poultry feed industry to select high quality feed ingredients (high in available nutrient concentration and low in anti-nutrient contents) and to formulate economically efficient diets. Precise data on digestible AA levels in feed ingredients (field beans for example) for broilers allows the poultry feed industry to optimise the efficiency of diet preparation and minimises overfeeding of AAs, thereby optimising broiler performance and minimising feed cost (Tahir and Pesti, 2012).

Field beans are a high protein feed that additionally contain a high amount of starch. A high inclusion rate (for example 30% of diet) in typical UK broiler feeds would result in them replacing a significant proportion of the SBM and a proportion of the wheat

component. Therefore it is logical to compare the nutrient content of beans to these two feed ingredients.

The results from the first experiment in this project showed that field beans, in comparison to the published values (Ravindran *et al.,* 2014) for dehulled SBM, have approximately half of the protein concentration (282 g/kg DM *versus* 530 g/kg DM) with a similar variability between different batches (ranges of 60 g/kg DM and 65 g/kg DM). The quality of the protein from field beans compares favourably with SBM: When AA composition is expressed as a proportion of the protein supplied, field beans are a richer source of lysine (by 9 g/kg CP) and arginine (by 18 g/kg CP) compared to SBM. Although field beans have a lower content of the two sulphur containing AAs (methionine 7 g/kg CP and cysteine 2 g/kg CP lower) and the two aromatic amino acids (tyrosine 14 g/kg CP and phenylalanine 5 g/kg CP lower) this would not necessarily cause any problems in practical feed formulations for broiler chickens. Unlike SBM, field beans are unprocessed and, most importantly, are not subjected to any heat treatment. This appears to give some advantages to field beans: The variability in lysine concentration (a heat labile AA) between different cultivars of field beans was lower than that observed in SBM. The bioavailablility of lysine was numerically higher in field beans than the mean for different batches of SBM (Ravindran *et al.,* 2014). The relatively low protein concentration of field beans will make it unlikely that practical UK broiler diet formulations would ever be able to completely replace SBM as the protein concentrate, but it is possible that a significant proportion could be replaced with little or no deleterious effect on the quality or digestibility of the AA supply.

The low ME content of field beans is a major problem for their use in practical broiler feeds in the UK. Field beans would need to replace a proportion of the cereal (wheat) component of the diets, yet this study has shown that the mean AME of beans was 10.1 MJ/kg DM compared to a mean of 13.9 MJ/kg DM for wheat (Ball *et al.,* 2013) and only similar to the AME of SBM (10.0 MJ/kg DM). The variability of AME in field beans was similar to that of different batches of wheat (Ball *et al.,* 2013). Although beans have a relatively high starch content (mean of 456 with a range of 397-517 g/kg DM compared to 644 with a range of 567-719 g/kg DM for wheat) (Ball *et al.,* 2013), it also has a high fibre content. The mean I-NSP content was 131 g/kg DM and S-NSP was 51 g/kg DM and both these components had high variability in field beans. This high fibre content results in an overall low energy density for field beans. Economically efficient broiler feeds need to have high energy density and the low energy density of field beans is a major limitation to their widespread use in the UK broiler feed industry.

The seed-coat of the bean comprises 13.4% of its total weight (range of 11.7-14.9%) (Duc *et al.,* 1999). This component has the major concentration of the insoluble and soluble NSPs. It is technically feasible to dehull field beans and, although it was not part of the present project, Nalle *et al*. (2010b) reported that the AMEn of the dehulled beans was 12.06 MJ/kg DM. Commercial dehulling of field beans for animal feeding has not been undertaken in the UK, most probably primarily because of the problems and costs of disposing of the hulls. However, there has been a recent increase in the number of anaerobic digesters in the UK that use agricultural waste products as their feedstock (Defra, 2014). It is likely that field bean hulls could be sold as a co-product to use in anaerobic digesters and a nutritionally superior dehulled field bean product would then be available to the poultry feed industry. Lacassagne *et al.* (1991) found that AMEn of T+ and T- field bean culivars can be increased to 14.94 and 13.49 MJ/kg DM, respectively, by dehulling followed by fine grinding (passing through 0.16 mm mesh).

8. 2. Rapid tests of nutritional value between field bean samples

The previous section has discussed that there is variation in the nutritional value of different field bean samples. If field beans are to be used by the animal feed industry, then the buyers of loads of field beans for the feed compounders would need the ability to be able to select the batches that have the higher nutritional value. Any quality test carried out on these samples needs to be rapid.

The results from this project have shown that the single most useful characteristic that could be measured was seed colour: Lighter samples with higher L* scores (L* WB around 50 and L* FB above 93) indicated higher available energy and nutrient utilisation. There is general agreement that pale legume seeds have a higher nutritive value than dark seeded cultivars (Duc *et al.,* 1995). Greater darkness in field beans indicates high levels of condensed tannins, phytate, and fibres (Duc *et al.,* 1999; Oomah *et al.,* 2011) and is associated with relatively low energy and nutrient availability for poultry (Vilariño *et al.,* 2009). Although the colour of the seed-coat is genetically determined, it may also change depending on growing location, season and storage conditions (Beninger *et al.,* 2005; Oomah *et al.,* 2011). It has been reported that reduced tannin content in beans correlates to an increase in the cotyledon to seed-coat ratio, reduced NSP and increased CP contents (Helsper *et al.,* 1993; Duc *et al.,* 1999). It should be noted however, that in plants with coloured flowers, the seed colour in individual plants is not homogeneous, and light and dark beans can always be obtained (Marbach and Mayer, 1974). Unfortunately, little data on colour scoring of protein sources and their nutritive value is available. Overall, our results indicate that field beans with higher lightness values have greater energy and nutrient availability, and this is in agreement with previous observations with

peas, wheat and corn DDGS and milk powder (Igbasan *et al.,* 1997; van Boekel, 1998; Pedersen *et al.,* 2007; Cozannet *et al.,* 2010). Thus, the use of field beans with lighter colour, high cotyledon to seed-coat ratio, and high CP contents can be recommended for poultry diet formulation.

8. 3. Can the nutritional value of field beans be improved?

A major aspect of this project was to examine whether there are strategies that can be adopted that could improve the nutritional value of field beans for broiler chickens and/or reduce the variability of nutritional value between different batches. Identifying and understanding the effects of any improvements would then have a major impact on the economic value of field beans to the UK.

The relatively large NSP components of field beans have a major effect of reducing the AME of the feedstuff and so it was expected that enzymes that may degrade these components may be beneficial. Commercial xylanases and phytases are available at economically effective prices to the UK animal feed industry. The protein supply of beans is also important and commercial proteases are also available. Examination of the effects of these enzymes, singly or in combination, on three different field bean samples formed the basis of the second experiment in the present project. The three chosen bean samples had close protein contents, but different NSP and phytate contents. Addition of exogenous phytase alone improved dietary protein utilisation, and the addition of xylanase alone increased dietary dry mater and energy utilisation, while protease supplementation did not give any alteration in the measured variables. The observed improvements were similar in all diets and there was no evidence that they were able to reduce the variability in nutrient utilisation between the three different bean samples. In fact, the field beans with the highest protein and lowest NSPs and phytate contents tended to respond better to exogenous enzyme supplementation. The overall conclusion from this study was that, although commercially available xylanase, phytase and protease may be able to improve the nutrient availability of practical diets, they had no special value in improving the nutritional value of diets with a high field bean inclusion rate (300 g/kg diet). It is a possibility that not enough exogenous enzymes were added to the diets to have an effect. However, the enzymes were used at rates that were possibly economically viable and so it is unlikely that, even if very much higher exogenous enzyme inclusions gave some biological advantage, very high inclusion levels would be economically advantageous.

The second phase of the exogenous enzyme work involved exploring whether there may be commercially produced enzymes that are not normally used in proprietary broiler chicken feeds, but that could benefit diets with a high field bean inclusion. The results of the first feeding experiment indicated that there may be a negative relationship between

the tannin content and nutrient availability of the bean samples. Tannin is known to have major effects on protein and AA digestibility in poultry (Ortiz *et al.,* 1993; Vilariño *et al.,* 2009; Woyengo and Nyachoti, 2012). A commercial enzyme product with high tannase activity is used by the human food industry in the UK (Boadi and Neufeld, 2001; Aguilar *et al.,* 2007) and a preliminary experiment (feeding experiment 3) indicated that it could improve nutrient availability in a diet with a high inclusion rate of a high-tannin field bean sample. Addition of this enzyme product reduced pancreas weights in the broiler chickens, further suggesting that its mode of action may have been its ability to degrade tannin in the feed material and so require less pancreatic enzyme secretions. Feeding experiment 4 examined the use of this tannase enzyme in detail. The results of this experiment indicated that the improvement in nutritional value due to the addition of this exogenous enzyme mixture was not specific to the diets containing field beans, and a similar magnitude of response was obtained in non-bean containing, low tannin diets. The responses seemed unlikely to be due to the tannase and more likely due to the other enzymes (pectinase and amylase) that it contained. Pectinase and xylanase reduce digesta viscosity and increase the action of enzymes and their accessibility to their substrates, thus increasing the digestibility of dietary nutrients and absorption of the end products of digestion. Reduced digesta viscosity also increases the mobility of digesta through the gut, thereby reducing the digesta transit time and improving dietary nutrient utilisation. It is unlikely that the addition of this enzyme mixture to practical broiler feeds would ever be economically feasible. However, field bean breeders are able to reduce/ eliminate tannin content in bean cultivars via breeding and employing zero tannin genes, thus it can be expected that tannin containing beans will progressively disappear from the market. In addition, selection towards tannin reduction in beans will lead to reduced proportion of seed-coat, NSP and other ANFs, and increase CP content (Helsper *et al.,* 1993; Duc *et al.,* 1999).

The fifth feeding experiment reported in this project examined the possibility that heat treatment (micronising) of field beans could improve their nutritional value. Also, to investigate whether the combination of micronising and addition of the enzyme mixture containing tannase would lead to further improvement in the nutritional value of field beans for broiler chickens compared to enzyme supplementation only. Heat treatment of the beans reduced the degradability of condensed tannins but otherwise had no other beneficial effects on nutrient availability. Also, the combination of micronising and addition of the enzyme mixture containing tannase did not result in further improvement in the nutritional value of field beans for broiler chickens compared to enzyme supplementation only. Micronising of field beans requires initial grinding and the additional heating stage

before incorporation in the diets. It is unlikely that the additional costs would be justified economically.

In summary, there is a continued political will for greater food security and the greater use of home grown feedstuffs in the UK broiler production industry. Field bean production in the UK has increased markedly in recent years and its relatively high protein content and good protein quality makes it a possible feed ingredient to be used by the commercial broiler feed industry. The low AME of beans is the major problem in using greater proportions in practical broiler feed formulations. The present project has been unable to identify an enzyme or enzyme combination that could ecconomically improve the AME of the feedstuff. Future studies should focus on practical methods to dehull beans and processes that could find an economically viable use for the waste products from this.

8. 4. General conclusions

- The mean CP content of a range of UK field bean samples was 282 g/kg DM and the AME was 10.1 MJ/kg DM. These mean values should be used by nutritionists when formulating practical broiler feeds. The between batch variability of CP and AME was similar to other similar feedstuffs (SBM/wheat). The variability estimates can be used by nutritionists when they are using stochastic feed formulation methods.
- The AA composition of the protein in field beans is similar to that of SBM, although somewhat higher in base amino acids (lysine and arginine) and lower in sulphur containing (methionine and cysteine) and aromatic (phenylalanine and tyrosine) AAs. Amino acid availability was similar to that reported for SBM. The lack of any heat treatment for field beans probably results in less variability in important heat labile AAs such as lysine.
- There can be significant differences in the nutritional value of different field bean samples that are available to the poultry feed industry. Field bean cultivars with high protein content, cotyledon ratio and lightness score, and low NSPs are nutritionally superior for broilers. These characteristics could be used to select field bean samples for use in broiler feeds.
- The supplementation of exogenous phytase, xylanase and protease alone or in a combination did not improve nutrient utilisation of field beans for broilers to an economically important level.
- Supplementation of an enzyme mixture that had tannase, α-amylase, xylanase, and pectinase activities did not specifically enhance energy and nutrient utilisation of field beans in chickens.

- Effect of supplementation of pure tannase on the nutritional value of field beans for broilers should be investigated.
- Application of heat treatment (micronising) did not improve the nutritional value of field beans.

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APPENDICES

Appendix 1

Determination of trypsin inhibitors

Trypsin inhibitor concentration in field bean samples was determined by applying the method described by Smith *et al.* (1980).

Required solutions:

Tris buffer

Tris (50 mM, pH 8.2) containing 20 mM Calcium Chloride was prepared as follows: Exactly 6.05 g of (hydroxymethyl) methylamine and 2.94 g of $CaCl_2$. $2H_2O$ were dissolved in 900 ml deionised distilled water. The pH of the solution was then adjusted to 8.2 with HCl, and was checked after dilution to 1 litre.

BAPNA substrate

Exactly 40 mg of Benzoyl-DL-arginine-p-nitroanilide hydrochloride (BDH, Poole) was dissolved in 1 ml dimethyl sulphoxide. The solution was diluted to 100 ml with Tris buffer that was previously warmed to 37^{o}C, and kept at 37^{o}C while in use. Freshly prepared reagent was used each time.

Standard trypsin solution preparation

Exactly 40 mg of crystalline bovine trypsin (Boehrineger, Bell Lane, Lewes) was dissolved in 1 mM HCl, and the volume was made up to 2 litres with the acid solution. Reading of 2 ml of the solution (40 µg trypsin) gave an absorbance of 0.410 ± 0.010 after subtraction of the reagent blank.

Procedure

Extraction

1- One gram of a freshly ground (passing 0.8 mm sieve) and air-dried representative sample was taken into a 100 ml plastic cup, the volume was then made up to exactly 50 ml with 10 mM NaOH.
2- After tightly locking, the cop was shaken briefly for 5 minutes, following that the pH of the resulting slurry was adjusted to between 9.4-9.6 with 1 M NaOH or 1 M HCl while the slurry was being stirred on a magnetic stirrer.
3- The resulting slurry was left at 4^{o}C overnight.

4- After extraction, the suspension was shaken and diluted (D times) with deionized distilled water so that 1 ml produced trypsin inhibition (AI) of between 40 and 60%. Dilution factors (D) of about 3 and 3.5 for Arthur and Clipper respectively, 2.5 for Buzz and Sultan, 4 for Divine, Fury, Honey, Maris Bead and Wizard and 5 for Fuego field bean samples were prepared.

Determination

1- The following additions were pipetted into a series of 10-ml tubes in triplicate each run:

(i) *Reagent blank*: Two ml deionised distilled water.

(ii) Standard (40 µg trypsin): Two ml standard trypsin solution and 2 ml deionised distilled water.

(iii) Sample blank: one ml diluted sample extract and 1 ml deionised distilled water.

(iv) Sample: one ml diluted sample extract, 1 ml deionised distilled water and 2 ml standard trypsin solution.

2- After vortexing the tube contents and heating to 37^{o}C for 10 minutes, 5 ml BAPNA solution (previously warmed to 37^{o}C) was pipetted into each tube and vortexed.

3- Tubes were incubated for exactly 10 minutes at 37^{o}C, and 1 ml acetic acid (30% v/v) was then added to each tube to stop the reaction.

4- Two ml standard trypsin was then added to the reagent blank (i) and sample blank (iii) tubes.

5- The absorbance of the clear supernatant was measured at 410 nm after centrifugation (Sigma 3-16KL, Sigma Laborzentrifugen, GmbH, Germany) of the tube contents at 3000 x*g* at 20^{o}C for 5 minutes. The trypsin inhibitor activity was then determined using the following equation.

$$\text{TAI (mg pure trypsin inhibited/g sample)} = \frac{2.632 \times \text{D} \times A_I}{\text{Sample weigt (g)}}$$

When $A_I = (A_{ii}-A_i) - (A_{vi}-A_{iii})$, and $\frac{A_I}{(A_{ii}-A_i)} \times 100$ = (40-60%).

Where A is absorbance at 410 nm and the subscripts refer to tubes (i-iv) above.

Appendix 2

Coefficients of ileal fat (IFD) and dry matter (DMD) digestibility of diets containing 200 g/kg one of the ten different UK grown field bean cultivar samples fed to broiler chickens

Diets	IFD	DMD
Control + 20%Arthur	0.852	0.611[ab]
Control + 20%Buzz	0.819	0.591[ab]
Control + 20%Clipper	0.865	0.603[ab]
Control + 20%Divine	0.864	0.616[ab]
Control + 20%Fuego	0.854	0.620[b]
Control + 20%Fury	0.842	0.612[ab]
Control + 20%Honey	0.866	0.624[b]
Control + 20%Maris Bead	0.843	0.611[ab]
Control + 20%Sultan	0.859	0.584[a]
Control + 20%Wizard	0.843	0.622[b]
SEM (df=63)	0.0136	0.0075
P value	0.353	0.005

Each value represents mean of eight replicate pens of five birds each; Variables were determined from 21 days of age; SEM, standard error of the mean; [a,b]Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Appendix 3

Performance, and energy and nutrient digestibility of the experimental diets for broiler chickens

Diets	FI (g DM/b/d)	WG (g/b/d)	FCR	IFD	DMD
Control	54.4	45.3	1.199^{ab}	0.876	0.644^{b}
Control + 10%Honey	56.1	47.3	1.188^{a}	0.858	0.634^{b}
Control + 20%Arthur	57.4	46.2	1.244^{bcd}	0.852	0.611^{ab}
Control + 20%Buzz	59.3	47.3	1.254^{cd}	0.819	0.591^{a}
Control + 20%Clipper	58.3	47.0	1.239^{bcd}	0.865	0.603^{ab}
Control + 20%Divine	60.0	50.0	1.201^{ab}	0.864	0.616^{ab}
Control + 20%Fuego	57.7	46.9	1.233^{abcd}	0.854	0.620^{ab}
Control + 20%Fury	58.5	48.2	1.212^{abc}	0.842	0.612^{ab}
Control + 20%Honey	58.8	48.3	1.220^{abc}	0.866	0.624^{ab}
Control + 20%Maris Bead	59.5	48.7	1.221^{abc}	0.843	0.611^{ab}
Control + 20%Sultan	57.4	45.1	1.274^{d}	0.859	0.584^{a}
Control + 20%Wizard	56.5	46.4	1.217^{abc}	0.843	0.622^{ab}
SEM (df=77)	1.20	1.05	0.0104	0.0133	0.0085
P value	0.071	0.070	<0.001	0.264	<0.001

FI, daily feed intake; WG, daily weight gain; FCR, feed conversion ratio; IFD, coefficient of ileal fat digestibility; DMD, coefficient of ileal dry matter digestibility; Each value represents mean of eight replicate pens of five birds each; Bird performance was determined from 7 to 21 days of age; IFD and DMD variables were determined from 21 days of age; SEM, standard error of the mean; ^{a,b,c,d}Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Appendix 4. Apparent ileal digestibility coefficient of amino acids of diets containing 200 g/kg of one of the ten UK grown field bean cultivar samples fed to broiler chickens

Amino acid digestibility	Diets										SEM (df=63)	*P* value
	Control + 20% Arthur	Control + 20% Buzz	Control + 20% Clipper	Control + 20% Divine	Control + 20% Fuego	Control + 20% Fury	Control + 20% Honey	Control + 20% Maris Beads	Control + 20% Sultan	Control + 20% Wizard		
Indispensable amino acids												
Arginine	0.791^{bc}	0.771^{ab}	0.786^{abc}	0.804^{c}	0.789^{abc}	0.797^{bc}	0.812^{c}	0.810^{c}	0.762^{a}	0.810^{c}	0.0062	<0.001
Histidine	0.662^{ab}	0.662^{ab}	0.657^{ab}	0.687^{b}	0.656^{ab}	0.686^{b}	0.692^{b}	0.692^{b}	0.635^{a}	0.689^{b}	0.0088	<0.001
Isoleucine	0.725^{bc}	0.712^{abc}	0.709^{ab}	0.733^{bc}	0.715^{abc}	0.731^{bc}	0.738^{c}	0.728^{bc}	0.691^{a}	0.733^{bc}	0.0060	<0.001
Leucine	0.721^{bc}	0.710^{bc}	0.704^{ab}	0.731^{bc}	0.717^{bc}	0.727^{bc}	0.732^{c}	0.729^{bc}	0.682^{a}	0.729^{bc}	0.0059	<0.001
Lysine	0.789^{b}	0.762^{a}	0.772^{ab}	0.789^{b}	0.783^{ab}	0.785^{ab}	0.792^{b}	0.789^{b}	0.770^{ab}	0.792^{b}	0.0053	<0.001
Methionine	0.955	0.942	0.951	0.947	0.951	0.947	0.954	0.946	0.947	0.953	0.0039	0.322
Phenylalanine	0.761^{b}	0.741^{ab}	0.737^{ab}	0.757^{b}	0.742^{ab}	0.752^{ab}	0.761^{b}	0.753^{ab}	0.726^{a}	0.756^{b}	0.0058	<0.001
Threonine	0.707^{b}	0.694^{ab}	0.699^{b}	0.723^{b}	0.704^{b}	0.725^{b}	0.719^{b}	0.724^{b}	0.664^{a}	0.720^{b}	0.0072	<0.001
Valine	0.706^{b}	0.692^{ab}	0.694^{ab}	0.716^{b}	0.697^{b}	0.718^{b}	0.720^{b}	0.714^{b}	0.664^{a}	0.715^{b}	0.0067	<0.001
Dispensable amino acids												
Alanine	0.721^{bc}	0.699^{ab}	0.703^{abc}	0.728^{bc}	0.712^{bc}	0.726^{bc}	0.729^{c}	0.725^{bc}	0.682^{a}	0.726^{bc}	0.0063	<0.001
Aspartic acid	0.721^{b}	0.708^{b}	0.709^{b}	0.735^{b}	0.717^{b}	0.736^{b}	0.733^{b}	0.733^{b}	0.677^{a}	0.734^{b}	0.0065	<0.001
Cysteine	0.742^{bc}	0.730^{abc}	0.727^{ab}	0.756^{bc}	0.749^{bc}	0.743^{bc}	0.764^{c}	0.746^{bc}	0.700^{a}	0.737^{bc}	0.0078	<0.001
Glutamic acid	0.817^{b}	0.799^{ab}	0.804^{ab}	0.815^{b}	0.807^{b}	0.813^{b}	0.818^{b}	0.815^{b}	0.785^{a}	0.819^{b}	0.0045	<0.001
Glycine	0.677^{bc}	0.663^{abc}	0.662^{ab}	0.698^{c}	0.672^{bc}	0.696^{bc}	0.697^{c}	0.697^{c}	0.633^{a}	0.692^{bc}	0.0075	<0.001
Proline	0.720^{b}	0.722^{b}	0.714^{ab}	0.729^{b}	0.718^{ab}	0.722^{b}	0.736^{b}	0.723^{b}	0.688^{a}	0734^{b}	0.0067	<0.001
Serine	0.717^{b}	0.703^{ab}	0.705^{b}	0.733^{b}	0.720^{b}	0.727^{b}	0.730^{b}	0.732^{b}	0.674^{a}	0.729^{b}	0.0065	<0.001
Tyrosine	0.708^{abcd}	0.702^{abc}	0.711^{abcd}	0.734^{d}	0.694^{ab}	0.722^{bcd}	0.726^{cd}	0.721^{bcd}	0.691^{a}	0.715^{abcd}	0.0063	<0.001
Indispensable amino acids	0.755^{bc}	0.739^{ab}	0.742^{abc}	0.763^{bc}	0.744^{abc}	0.759^{bc}	0766^{c}	0.763^{bc}	0.724^{a}	0.764^{bc}	0.0057	<0.001
Dispensable amino acids	0.751^{b}	0.738^{ab}	0.737^{ab}	0.760^{b}	0.745^{b}	0.757^{b}	0.761^{b}	0.757^{b}	0.718^{a}	0.759^{b}	0.0054	<0.001
Total amino acids	0.753^{b}	0.738^{ab}	0.740^{ab}	0.761^{b}	0.745^{b}	0.758^{b}	0.763^{b}	0.760^{b}	0.716^{a}	0.761^{b}	0.0056	<0.001

Each value represents mean of eight replicate pens of five birds each; Variables were determined at 21 days of age; SEM, standard error of the mean; ^{a,b,c,d}Values within a row with different superscripts differ significantly at $P \leq 0.05$.

Appendix 5. Apparent ileal digestibility coefficient of amino acids of the experimental diets fed to broiler chickens

Amino acid digestibility	Diets												SEM (df=77)	*P* value
	Control	Control + 10% Honey	Control + 20% Arthur	Control + 20% Buzz	Control + 20% Clipper	Control + 20% Divine	Control + 20% Fuego	Control + 20% Fury	Control + 20% Honey	Control + 20% Maris Beads	Control + 20% Sultan	Control + 20% Wizard		
Indispensable amino acids														
Arginine	0.750^{a}	0.789^{bcd}	0.791^{bcd}	0.771^{abc}	0.786^{bcd}	0.804^{d}	0.789^{bcd}	0.797^{cd}	0.812^{d}	0.810^{d}	0.762^{ab}	0.810^{d}	0.0066	<0.001
Histidine	0.597^{a}	0.665^{bc}	0.662^{bc}	0.662^{bc}	0.657^{bc}	0.687^{c}	0.656^{bc}	0.686^{c}	0.692^{c}	0.692^{c}	0.635^{ab}	0.689^{c}	0.0096	0.005
Isoleucine	0.662^{a}	0.717^{bc}	0.725^{bc}	0.712^{bc}	0.709^{bc}	0.733^{c}	0.715^{bc}	0.731^{c}	0.738^{c}	0.728^{c}	0.691^{ab}	0.733^{c}	0.0073	<0.001
Leucine	0.671^{a}	0.717^{c}	0.721^{c}	0.710^{bc}	0.704^{bc}	0.731^{c}	0.717^{c}	0.727^{c}	0.732^{c}	0.729^{c}	0.682^{ab}	0.729^{c}	0.0065	<0.001
Lysine	0.728^{a}	0.777^{b}	0.789^{b}	0.762^{b}	0.772^{b}	0.789^{b}	0.783^{b}	0.785^{b}	0.792^{b}	0.789^{b}	0.770^{b}	0.792^{b}	0.0063	<0.001
Methionine	0.965^{b}	0.959^{ab}	0.955^{ab}	0.942^{a}	0.951^{ab}	0.947^{a}	0.951^{ab}	0.947^{a}	0.954^{ab}	0.946^{a}	0.946^{a}	0.953^{ab}	0.0037	0.003
Phenylalanine	0.726^{ab}	0.754^{abc}	0.761^{c}	0.741^{abc}	0.737^{abc}	0.757^{bc}	0.742^{abc}	0.752^{abc}	0.761^{c}	0.753^{abc}	0.726^{a}	0.756^{abc}	0.0064	<0.001
Threonine	0.656^{a}	0.706^{c}	0.707^{c}	0.694^{bc}	0.699^{bc}	0.723^{c}	0.705^{c}	0.725^{c}	0.719^{c}	0.724^{c}	0.664^{ab}	0.720^{c}	0.0079	<0.001
Valine	0.633^{a}	0.693^{bc}	0.706^{c}	0.692^{bc}	0.694^{bc}	0.716^{c}	0.697^{bc}	0.718^{c}	0.720^{c}	0.714^{c}	0.664^{ab}	0.715^{c}	0.0077	<0.001
Dispensable amino acids														
Alanine	0.651^{a}	0.705^{bc}	0.721^{c}	0.699^{bc}	0.703^{bc}	0.728^{c}	0.712^{bc}	0.726^{c}	0.729^{c}	0.725^{c}	0.682^{ab}	0.726^{c}	0.0073	<0.001
Aspartic acid	0.629^{a}	0.703^{bc}	0.721^{c}	0.708^{bc}	0.709^{bc}	0.735^{c}	0.717^{c}	0.736^{c}	0.733^{c}	0.733^{c}	0.677^{b}	0.734^{c}	0.0073	<0.001
Cysteine	0.709^{ab}	0.736^{abc}	0.742^{bc}	0.730^{abc}	0.727^{abc}	0.756^{c}	0.749^{c}	0.743^{bc}	0.764^{c}	0.746^{bc}	0.700^{a}	0.737^{abc}	0.0081	<0.001
Glutamic acid	0.794^{ab}	0.816^{bc}	0.817^{bc}	0.799^{abc}	0.804^{abc}	0.815^{bc}	0.807^{abc}	0.813^{bc}	0.818^{c}	0.815^{bc}	0.785^{a}	0.819^{c}	0.0050	<0.001
Glycine	0.590^{a}	0.657^{bc}	0.677^{c}	0.663^{bc}	0.662^{bc}	0.698^{c}	0.672^{bc}	0.696^{c}	0.697^{c}	0.697^{c}	0.633^{b}	0.692^{c}	0.0088	<0.001
Proline	0.752^{d}	0.748^{cd}	0.720^{abcd}	0.722^{bcd}	0.714^{ab}	0.729^{bcd}	0.718^{abc}	0.722^{bcd}	0.736^{bcd}	0.723^{bcd}	0.688^{a}	0734^{bcd}	0.0069	<0.001
Serine	0.659^{a}	0.710^{c}	0.717^{c}	0.703^{bc}	0.705^{bc}	0.733^{c}	0.720^{c}	0.727^{c}	0.730^{c}	0.732^{c}	0.674^{ab}	0.729^{c}	0.0070	<0.001
Tyrosine	0.708^{abc}	0.729^{c}	0.708^{abc}	0.702^{abc}	0.711^{abc}	0.734^{c}	0.694^{ab}	0.722^{abc}	0.726^{bc}	0.721^{abc}	0.691^{a}	0.715^{abc}	0.0069	<0.001
Indispensable amino acids	0.711^{a}	0.751^{bc}	0.755^{c}	0.739^{abc}	0.742^{bc}	0.763^{c}	0.744^{bc}	0.759^{c}	0766^{c}	0.763^{c}	0.724^{ab}	0.764^{c}	0.0064	<0.001
Dispensable amino acids	0.715^{a}	0.749^{c}	0.751^{c}	0.738^{abc}	0.737^{abc}	0.760^{c}	0.745^{bc}	0.757^{c}	0.761^{c}	0.757^{c}	0.718^{ab}	0.759^{c}	0.0061	<0.001
Total amino acids	0.713^{a}	0.750^{c}	0.753^{c}	0.738^{abc}	0.740^{abc}	0.761^{c}	0.745^{bc}	0.758^{c}	0.763^{c}	0.760^{c}	0.716^{ab}	0.761^{c}	0.0062	<0.001

Each value represents mean of eight replicate pens of five birds each; Variables were determined at 21 days of age; SEM, standard error of the mean; ^{a,b,c,d}Values within a row with different superscripts differ significantly at $P \leq 0.05$.

Appendix 6

Energy and nutrient availability of diets containing 200 g/kg of one of ten UK grown field bean cultivar samples fed to broiler chickens

Diets	AME (MJ/kg DM)	AMEn (MJ/kg DM)	NR	FD	DMR
Control + 20%Arthur	14.13^{abc}	13.25^{abc}	0.619	0.750	0.682^{ab}
Control + 20% Buzz	13.93^{ab}	13.06^{ab}	0.611	0.747	0.671^{ab}
Control + 20%Clipper	14.14^{abc}	13.25^{abc}	0.619	0.780	0.678^{ab}
Control + 20%Divine	14.32^{c}	13.41^{c}	0.625	0.782	0.687^{b}
Control + 20%Fuego	14.25^{bc}	13.37^{bc}	0.621	0.771	0.685^{b}
Control + 20%Fury	14.26^{bc}	13.38^{bc}	0.614	0.773	0.686^{b}
Control + 20%Honey	14.20^{bc}	13.30^{abc}	0.621	0.771	0.683^{b}
Control + 20%Maris Bead	14.18^{abc}	13.29^{abc}	0.612	0.770	0.684^{b}
Control + 20%Sultan	13.82^{a}	12.97^{a}	0.608	0.769	0.665^{a}
Control + 20%Wizard	14.21^{bc}	13.32^{bc}	0.611	0.775	0.682^{ab}
SEM (df=63)	0.078	0.074	0.0042	0.0083	0.0037
P value	<0.001	<0.001	0.091	0.061	0.001

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy, FD, coefficient of total tract fat digestibility; NR, coefficient of total tract nitrogen retention; DMR, coefficient of total tract dry matter retention; Each value represents mean of eight replicate pens of five birds each; Variables were determined from 17 to 21 days age; SEM, standard error of the mean; ^{a,b,c}Values within a column with different superscripts differ significantly at $P \leq 0.05$.

Appendix 7

Energy and nutrient availability of the experimental diets fed to broiler chickens

Diets	AME (MJ/kg DM)	AMEn	NR	FD	DMR
Control	15.15^{c}	14.27^{d}	0.625	0.749	0.716^{d}
Control + 10%Honey	14.75^{c}	13.86^{c}	0.623	0.756	0.702^{cd}
Control + 20%Arthur	14.13^{ab}	13.25^{ab}	0.619	0.750	0.682^{ab}
Control + 20%Buzz	13.93^{ab}	13.06^{ab}	0.611	0.747	0.671^{ab}
Control + 20%Clipper	14.15^{ab}	13.26^{ab}	0.619	0.781	0.679^{ab}
Control + 20%Divine	14.32^{b}	13.41^{b}	0.625	0.782	0.687^{bc}
Control + 20%Fuego	14.25^{b}	13.37^{b}	0.621	0.771	0.685^{abc}
Control + 20%Fury	14.26^{b}	13.38^{b}	0.614	0.773	0.686^{bc}
Control + 20%Honey	14.20^{ab}	13.30^{ab}	0.621	0.771	0.683^{abc}
Control + 20%Maris Bead	14.18^{ab}	13.29^{ab}	0.612	0.770	0.684^{abc}
Control + 20%Sultan	13.82^{a}	12.97^{a}	0.608	0.769	0.665^{a}
Control + 20%Wizard	14.21^{ab}	13.32^{ab}	0.611	0.775	0.682^{ab}
SEM (df=77)	0.087	0.083	0.0042	0.0093	0.0042
P value	<0.001	<0.001	0.038	0.073	<0.001

AME, apparent metabolisable energy; AMEn, nitrogen-corrected apparent metabolisable energy, FD, coefficient of total tract fat digestibility; NR, coefficient of total tract nitrogen retention; DMR, coefficient of total tract dry matter retention; Each value represents mean of eight replicate pens of five birds each; Variables were determined from 17 to 21 days of age; SEM, standard error of the mean; ^{a,b,c}Values within a column with different superscripts differ significantly at $P \leq 0.05$.